

65	HOUFS04	182	<p>Activation of transcription through NFKB response element in immune cells (such as T-cells).</p>	<p>Assays for the activation of transcription through the NFKB response element are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate NFKB transcription factors and modulate expression of immunomodulatory genes. Exemplary assays for transcription through the NFKB response element that may be used or routinely modified to test NFKB-response element activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); Black et al., Virus Gnes 15(2):105-117 (1997); and Fraser et al., 29(3):838-844 (1999), the contents of each of which are herein incorporated by reference</p>	<p>Highly preferred indications include inflammation and inflammatory disorders. Highly preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Highly preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below), and immunodeficiencies (e.g., as described below). An additional highly preferred indication is infection (e.g., AIDS, and/or an infectious disease as described below under "Infectious Disease"). Highly preferred indications include neoplastic diseases (e.g., melanoma, leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Highly preferred indications include neoplasms and cancers, such as, for example, melanoma, renal cell carcinoma, leukemia, lymphoma, and prostate, breast, lung, colon, pancreatic,</p>
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				in its entirety. Exemplary human T cells, such as the MOLT4, that may be used according to these assays are publicly available (e.g., through the ATCC).	esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications also include anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, suppression of immune reactions to transplanted organs, asthma and allergy.
66	HOUHI25	183	Activation of transcription through STAT6 response element in immune cells (such as T-cells).	Assays for the activation of transcription through the Signal Transducers and Activators of Transcription (STAT6) response element are well-known in the art and may be used or routinely modified to assess the ability of	<p>A highly preferred indication is allergy. Another highly preferred indication is asthma. Additional highly preferred indications include inflammation and inflammatory disorders. Preferred</p>

				<p>polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate STAT6 transcription factors and modulate the expression of multiple genes. Exemplary assays for transcription through the STAT6 response element that may be used or routinely modified to test STAT6 response element activity of the polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); Georas et al., Blood 92(12):4529-4538 (1998); Moffatt et al., Transplantation 69(7):1521-1523 (2000); Curiel et al., Eur J Immunol 27(8):1982-1987 (1997); and Masuda et al., J Biol Chem 275(38):29331-29337 (2000), the contents of each of which are herein incorporated by reference in its entirety. T cells that may be used according</p>	<p>indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, melanoma, and/or as described below under "Hyperproliferative Disorders"). Preferred indications include neoplasms and cancers, such as, leukemia, lymphoma, melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include anemia, pancytopenia,</p>
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				<p>to these assays are publicly available (e.g., through the ATCC). Exemplary T cells that may be used according to these assays include the SUPT cell line, which is a suspension culture of IL-2 and IL-4 responsive T cells.</p>	<p>leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, and Lyme Disease. An additional preferred indication is infection (e.g., an infectious disease as described below under "Infectious Disease").</p>
67	HPCAL26	184	<p>Activation of transcription through GAS response element in immune cells (such as T-cells).</p>	<p>Assays for the activation of transcription through the Gamma Interferon Activation Site (GAS) response element are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate STAT transcription factors and</p>	<p>Highly preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Highly preferred indications include neoplasms and cancers, such as, for example, leukemia, lymphoma (e.g., T cell lymphoma, Burkitt's lymphoma, non-Hodgkins lymphoma, Hodgkin's disease),</p>

				<p>modulate gene expression involved in a wide variety of cell functions. Exemplary assays for transcription through the GAS response element that may be used or routinely modified to test GAS-response element activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); Matikainen et al., Blood 93(6):1980-1991 (1999); and Henttinen et al., J Immunol 155(10):4582-4587 (1995), the contents of each of which are herein incorporated by reference in its entirety. Exemplary mouse T cells that may be used according to these assays are publicly available (e.g., through the ATCC). Exemplary T cells that may be used according to these assays include the CTL cell line, which is a suspension culture of IL-2 dependent</p>	<p>melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response. Additional preferred indications include inflammation and inflammatory disorders. Highly preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"), and infection (e.g., viral infections, tuberculosis, infections associated with chronic granulomatous disease</p>
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				cytotoxic T cells.	and malignant osteoporosis, and/or an infectious disease as described below under "Infectious Disease"). An additional preferred indication is idiopathic pulmonary fibrosis. Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, and asthma and allergy.
67	HPCAL26	184	Production of IL-10 and activation of T-cells.	Assays for production of IL-10 and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit	Highly preferred indications include allergy and asthma. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid

				<p>production of IL-10 and/or activation of T-cells. Exemplary assays that may be used or routinely modified to assess the ability of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) to modulate IL-10 production and/or T-cell proliferation include, for example, assays such as disclosed and/or cited in: Robinson, DS, et al., "Th-2 cytokines in allergic disease" Br Med Bull; 56 (4): 956-968 (2000), and Cohn, et al., "T-helper type 2 cell-directed therapy for asthma" Pharmacology & Therapeutics; 88: 187-196 (2000); the contents of each of which are herein incorporated by reference in their entirety. Exemplary cells that may be used according to these assays include Th2 cells. IL10 secreted from Th2 cells may be measured as a marker of Th2 cell activation. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6. Factors that induce differentiation and activation of Th2 cells play a major role in</p>	<p>arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.</p>
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				the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated via in vitro culture under Th2 polarizing conditions using peripheral blood lymphocytes isolated from cord blood.	
68	HPEBA84	185	Production of IL-10 and activation of T-cells.	Assays for production of IL-10 and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-10 and/or activation of T-cells. Exemplary assays that may be used or routinely modified to assess the ability of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) to modulate IL-10 production and/or T-cell proliferation include, for example, assays such as disclosed and/or cited in: Robinson, DS, et al., "Th-2 cytokines in allergic disease" Br Med Bull; 56 (4): 956-968 (2000), and Cohn, et al., "T-	Highly preferred indications include allergy and asthma. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.

69	HSAVA08	186	<p>Activation of transcription through serum response element in immune cells (such as T-cells).</p>	<p>Assays for the activation of transcription through the Serum Response Element (SRE) are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and</p>	<p>helper type 2 cell-directed therapy for asthma" Pharmacology & Therapeutics; 88: 187-196 (2000); the contents of each of which are herein incorporated by reference in their entirety. Exemplary cells that may be used according to these assays include Th2 cells. IL10 secreted from Th2 cells may be measured as a marker of Th2 cell activation. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6. Factors that induce differentiation and activation of Th2 cells play a major role in the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated via in vitro culture under Th2 polarizing conditions using peripheral blood lymphocytes isolated from cord blood.</p>	<p>A preferred embodiment of the invention includes a method for inhibiting (e.g., reducing) TNF alpha production. An alternative preferred embodiment of the invention includes a method for stimulating (e.g., increasing)</p>
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				<p>agonists or antagonists of the invention) to regulate the serum response factors and modulate the expression of genes involved in growth. Exemplary assays for transcription through the SRE that may be used or routinely modified to test SRE activity of the polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); and Black et al., Virus Genes 12(2):105-117 (1997), the content of each of which are herein incorporated by reference in its entirety. T cells that may be used according to these assays are publicly available (e.g., through the ATCC). Exemplary mouse T cells that may be used according to these assays include the CTLL cell line, which is an IL-2 dependent suspension culture of T cells with cytotoxic activity.</p>	<p>TNF alpha production. Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"), Highly preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response. Additional highly preferred indications include inflammation and inflammatory disorders, and treating joint damage in patients with rheumatoid arthritis. An additional highly preferred indication is sepsis. Highly preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Additionally, highly preferred indications include neoplasms</p>
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					<p>and cancers, such as, for example, leukemia, lymphoma, melanoma, glioma (e.g., malignant glioma), solid tumors, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, cardiac reperfusion injury, and asthma and allergy. An additional preferred indication is</p>
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69	HSA VA08	186	Production of IL-5	<p>IL-5 FMAT. Assays for immunomodulatory proteins secreted by TH2 cells, mast cells, basophils, and eosinophils that stimulate eosinophil function and B cell Ig production and promote polarization of CD4+ cells into TH2 cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to mediate immunomodulation, stimulate immune cell function, modulate B cell Ig production, modulate immune cell polarization, and/or mediate humoral or cell-mediated immunity. Exemplary assays that test for immunomodulatory proteins evaluate the production of cytokines, such as IL-5, and the stimulation of eosinophil function and B cell Ig production. Such assays that may be used or routinely</p>	<p>infection (e.g., an infectious disease as described below under "Infectious Disease").</p> <p>A highly preferred embodiment of the invention includes a method for inhibiting (e.g., reducing) IL-5 production. An alternative highly preferred embodiment of the invention includes a method for stimulating (e.g., increasing) IL-5 production. A highly preferred embodiment of the invention includes a method for stimulating (e.g., increasing) immunoglobulin production. An alternative highly preferred embodiment of the invention includes a method for inhibiting (e.g., decreasing) immunoglobulin production. A highly preferred indication includes allergy. A highly preferred indication includes asthma. A highly preferred indication includes rhinitis. An additional highly preferred indication is infection (e.g., an infectious disease as described below under "Infectious Disease"), and inflammation and inflammatory disorders.</p>
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			<p>modified to test immunomodulatory activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Miraglia et al., J Biomolecular Screening 4:193-204 (1999); Rowland et al., "Lymphocytes: a practical approach" Chapter 6:138-160 (2000); Ohshima et al., Blood 92(9):3338-3345 (1998); Jung et al., Eur J Immunol 25(8):2413-2416 (1995); Mori et al., J Allergy Clin Immunol 106(1 Pt 2):558-564 (2000); and Koning et al., Cytokine 9(6):427-436 (1997), the contents of each of which are herein incorporated by reference in its entirety. Human T cells that may be used according to these assays may be isolated using techniques disclosed herein or otherwise known in the art. Human T cells are primary human lymphocytes that mature in the thymus and express a T cell receptor and CD3, CD4, or CD8. These cells mediate humoral or cell-mediated immunity and may be</p>	<p>Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, melanoma, and/or as described below under "Hyperproliferative Disorders"). Preferred indications include neoplasms and cancers, such as, leukemia, lymphoma, melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include anemia, pancytopenia, leukopenia,</p>
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				preactivated to enhance responsiveness to immunomodulatory factors.	thrombocytopenia, leukemias, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, and Lyme Disease.
70	HSHAX04	187	Production of IL-10 and activation of T-cells.	Assays for production of IL-10 and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-10 and/or activation of T-cells. Exemplary assays that may be used or routinely modified to assess the ability of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) to modulate IL-10 production	Highly preferred indications include allergy and asthma. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.

71	Apoptosis	<p>caspase apoptosis are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to promote caspase protease-mediated apoptosis. Induction of apoptosis in endothelial cells supporting the vasculature of tumors is associated with tumor regression due to loss of tumor blood supply. Exemplary assays for caspase apoptosis that may be used or routinely modified to test caspase apoptosis activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Lee et al., FEBS Lett 485(2-3): 122-126 (2000); Nor et al., J Vasc Res 37(3): 209-218 (2000); and Karsan and Harlan, J Atheroscler Thromb 3(2): 75-80 (1996); the contents of each of which are herein incorporated by reference in its entirety. Endothelial cells that may be used according to these assays are publicly available</p>	<p>embodiment of the invention includes a method for stimulating endothelial cell growth. An alternative highly preferred embodiment of the invention includes a method for inhibiting endothelial cell growth. A highly preferred embodiment of the invention includes a method for stimulating endothelial cell proliferation. An alternative highly preferred embodiment of the invention includes a method for inhibiting endothelial cell proliferation. A highly preferred embodiment of the invention includes a method for stimulating apoptosis of endothelial cells. An alternative highly preferred embodiment of the invention includes a method for inhibiting (e.g., decreasing) apoptosis of endothelial cells. A highly preferred embodiment of the invention includes a method for stimulating angiogenesis. An alternative highly preferred embodiment of the invention includes a method for inhibiting angiogenesis. A highly preferred embodiment of the invention includes a</p>
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				<p>(e.g., through commercial sources). Exemplary endothelial cells that may be used according to these assays include bovine aortic endothelial cells (bAEC), which are an example of endothelial cells which line blood vessels and are involved in functions that include, but are not limited to, angiogenesis, vascular permeability, vascular tone, and immune cell extravasation.</p>	<p>method for reducing cardiac hypertrophy. An alternative highly preferred embodiment of the invention includes a method for inducing cardiac hypertrophy. Highly preferred indications include neoplastic diseases (e.g., as described below under “Hyperproliferative Disorders”), and disorders of the cardiovascular system (e.g., heart disease, congestive heart failure, hypertension, aortic stenosis, cardiomyopathy, valvular regurgitation, left ventricular dysfunction, atherosclerosis and atherosclerotic vascular disease, diabetic nephropathy, intracardiac shunt, cardiac hypertrophy, myocardial infarction, chronic hemodynamic overload, and/or as described below under “Cardiovascular Disorders”). Highly preferred indications include cardiovascular, endothelial and/or angiogenic disorders (e.g., systemic disorders that affect vessels such as diabetes mellitus, as well as diseases of the vessels</p>
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					<p>themselves, such as of the arteries, capillaries, veins and/or lymphatics). Highly preferred are indications that stimulate angiogenesis and/or cardiovascularization. Highly preferred are indications that inhibit angiogenesis and/or cardiovascularization.</p> <p>Highly preferred indications include antiangiogenic activity to treat solid tumors, leukemias, and Kaposi's sarcoma, and retinal disorders. Highly preferred indications include neoplasms and cancer, such as, Kaposi's sarcoma, hemangioma (capillary and cavernous), glomus tumors, telangiectasia, bacillary angiomatosis, hemangioendothelioma, angiosarcoma, haemangiopericytoma, lymphangioma, lymphangiosarcoma. Highly preferred indications also include cancers such as, prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver, and urinary cancer. Preferred indications include benign dysproliferative disorders and pre-neoplastic</p>
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					<p>conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Highly preferred indications also include arterial disease, such as, atherosclerosis, hypertension, coronary artery disease, inflammatory disease, vasculitides, Reynaud's disease and Reynaud's phenomenon, aneurysms, restenosis; venous and lymphatic disorders such as thrombophlebitis, lymphangitis, and lymphedema; and other vascular disorders such as peripheral vascular disease, and cancer. Highly preferred indications also include trauma such as wounds, burns, and injured tissue (e.g., vascular injury such as, injury resulting from balloon angioplasty, and atherosclerotic lesions), implant fixation, scarring, ischemia reperfusion injury, rheumatoid arthritis, cerebrovascular disease, renal diseases such as acute renal failure, and osteoporosis. Additional highly preferred indications include stroke, graft rejection, diabetic or other retinopathies, thrombotic and coagulative disorders,</p>
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					<p>vascularitis, lymph angiogenesis, sexual disorders, age-related macular degeneration, and treatment /prevention of endometriosis and related conditions. Additional highly preferred indications include fibromas, heart disease, cardiac arrest, heart valve disease, and vascular disease. Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Additional preferred indications include inflammation and inflammatory disorders (such as acute and chronic inflammatory diseases, e.g., inflammatory bowel disease and Crohn's disease), and pain management.</p> <p>Preferred indications include</p>
HSKDR27	188	Activation of T-Cell	Kinase assay. JNK and p38		

71	p38 or JNK Signaling Pathway.	<p>kinase assays for signal transduction that regulate cell proliferation, activation, or apoptosis are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to promote or inhibit immune cell (e.g. T-cell) proliferation, activation, and apoptosis. Exemplary assays for JNK and p38 kinase activity that may be used or routinely modified to test JNK and p38 kinase-induced activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Forrer et al., Biol Chem 379(8-9):1101-1110 (1998); Gupta et al., Exp Cell Res 247(2): 495-504 (1999); Kyriakis JM, Biochem Soc Symp 64:29-48 (1999); Chang and Karin, Nature 410(6824):37-40 (2001); and Cobb MH, Prog Biophys Mol Biol 71(3-4):479-500 (1999); the contents of each of which are herein incorporated by</p>	<p>neoplastic diseases (e.g., as described below under "Hyperproliferative Disorders"), blood disorders (e.g., as described below under "Immune Activity", "Cardiovascular Disorders", and/or "Blood-Related Disorders"), and infection (e.g., an infectious disease as described below under "Infectious Disease"). Highly preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Additional highly preferred indications include inflammation and inflammatory disorders. Highly preferred indications also include neoplastic diseases (e.g., leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Highly preferred indications include neoplasms and cancers, such as, leukemia, lymphoma, prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver, and urinary cancer.</p>
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				reference in its entirety. T cells that may be used according to these assays are publicly available (e.g., through the ATCC). Exemplary mouse T cells that may be used according to these assays include the CTLL cell line, which is an IL-2 dependent suspension-culture cell line with cytotoxic activity.	Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include arthritis, asthma, AIDS, allergy, anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, granulomatous disease, inflammatory bowel disease, sepsis, psoriasis, suppression of immune reactions to transplanted organs and tissues, endocarditis, meningitis, and Lyme Disease.
71	HSKDR27	188	Production of IL-4	IL-4 FMAT. Assays for immunomodulatory proteins secreted by TH2 cells that stimulate B cells, T cells, macrophages and mast cells and promote polarization of CD4+ cells into TH2 cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and	A highly preferred embodiment of the invention includes a method for stimulating (e.g., increasing) IL-4 production. An alternative highly preferred embodiment of the invention includes a method for inhibiting (e.g., reducing) IL-4 production. A highly preferred indication includes asthma. A highly preferred indication includes allergy. A

				<p>agonists or antagonists of the invention) to mediate immunomodulation, stimulate immune cells, modulate immune cell polarization, and/or mediate humoral or cell-mediated immunity. Exemplary assays that test for immunomodulatory proteins evaluate the production of cytokines, such as IL-4, and the stimulation of immune cells, such as B cells, T cells, macrophages and mast cells. Such assays that may be used or routinely modified to test immunomodulatory activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Miraglia et al., J Biomolecular Screening 4:193-204 (1999); Rowland et al., "Lymphocytes: a practical approach" Chapter 6:138-160 (2000); Gonzalez et al., J Clin Lab Anal 8(5):277-283 (1994); Yssel et al., Res Immunol 144(8):610-616 (1993); Bagley et al., Nat Immunol 1(3):257-261 (2000); and van der Graaff et al., Rheumatology (Oxford) 38(3):214-220 (1999), the</p>	<p>highly preferred indication includes rhinitis. Additional highly preferred indications include inflammation and inflammatory disorders. Highly preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, melanoma, and/or as described below under "Hyperproliferative Disorders"). Preferred indications include neoplasms and cancers, such as, for example, leukemia, lymphoma, melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic</p>
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				<p>contents of each of which are herein incorporated by reference in its entirety. Human T cells that may be used according to these assays may be isolated using techniques disclosed herein or otherwise known in the art. Human T cells are primary human lymphocytes that mature in the thymus and express a T cell receptor and CD3, CD4, or CD8. These cells mediate humoral or cell-mediated immunity and may be preactivated to enhance responsiveness to immunomodulatory factors.</p>	<p>lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, and Lyme Disease. An additional preferred indication is infection (e.g., an infectious disease as described below under "Infectious Disease").</p>
72	HSQBF66	189	Production of IL-5	<p>IL-5 FMAT. Assays for immunomodulatory proteins secreted by TH2 cells, mast cells, basophils, and eosinophils that stimulate eosinophil function and B cell Ig</p>	<p>A highly preferred embodiment of the invention includes a method for inhibiting (e.g., reducing) IL-5 production. An alternative highly preferred embodiment of the invention</p>

				<p>production and promote polarization of CD4+ cells into TH2 cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to mediate immunomodulation, stimulate immune cell function, modulate B cell Ig production, modulate immune cell polarization, and/or mediate humoral or cell-mediated immunity. Exemplary assays that test for immunomodulatory proteins evaluate the production of cytokines, such as IL-5, and the stimulation of eosinophil function and B cell Ig production. Such assays that may be used or routinely modified to test immunomodulatory activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Miraglia et al., J Biomolecular Screening 4:193-204 (1999); Rowland et al., "Lymphocytes: a practical</p>	<p>includes a method for stimulating (e.g., increasing) IL-5 production. A highly preferred embodiment of the invention includes a method for stimulating (e.g., increasing) immunoglobulin production. An alternative highly preferred embodiment of the invention includes a method for inhibiting (e.g., decreasing) immunoglobulin production. A highly preferred indication includes allergy. A highly preferred indication includes asthma. A highly preferred indication includes rhinitis. An additional highly preferred indication is infection (e.g., an infectious disease as described below under "Infectious Disease"), and inflammation and inflammatory disorders. Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple</p>
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			<p>approach" Chapter 6:138-160 (2000); Ohshima et al., Blood 92(9):3338-3345 (1998); Jung et al., Eur J Immunol 25(8):2413-2416 (1995); Mori et al., J Allergy Clin Immunol 106(1 Pt 2):558-564 (2000); and Koning et al., Cytokine 9(6):427-436 (1997), the contents of each of which are herein incorporated by reference in its entirety. Human T cells that may be used according to these assays may be isolated using techniques disclosed herein or otherwise known in the art. Human T cells are primary human lymphocytes that mature in the thymus and express a T cell receptor and CD3, CD4, or CD8. These cells mediate humoral or cell-mediated immunity and may be preactivated to enhance responsiveness to immunomodulatory factors.</p>	<p>sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, melanoma, and/or as described below under "Hyperproliferative Disorders"). Preferred indications include neoplasms and cancers, such as, leukemia, lymphoma, melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, leukemias, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, immune reactions to transplanted organs</p>
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73	HSRFD18	190	Production of IL-10 and activation of T-cells.	Assays for production of IL-10 and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-10 and/or activation of T-cells. Exemplary assays that may be used or routinely modified to assess the ability of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) to modulate IL-10 production and/or T-cell proliferation include, for example, assays such as disclosed and/or cited in: Robinson, DS, et al., "Th-2 cytokines in allergic disease" Br Med Bull; 56 (4): 956-968 (2000), and Cohn, et al., "T-helper type 2 cell-directed therapy for asthma" Pharmacology & Therapeutics;	and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, and Lyme Disease. Highly preferred indications include allergy and asthma. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.
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				<p>88: 187-196 (2000); the contents of each of which are herein incorporated by reference in their entirety. Exemplary cells that may be used according to these assays include Th2 cells. IL10 secreted from Th2 cells may be measured as a marker of Th2 cell activation. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6. Factors that induce differentiation and activation of Th2 cells play a major role in the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated via in vitro culture under Th2 polarizing conditions using peripheral blood lymphocytes isolated from cord blood.</p>	
75	HT3BF49	192	Production of IL-4	<p>IL-4 FMAT. Assays for immunomodulatory proteins secreted by TH2 cells that stimulate B cells, T cells, macrophages and mast cells and promote polarization of CD4+ cells into TH2 cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention</p>	<p>A highly preferred embodiment of the invention includes a method for stimulating (e.g., increasing) IL-4 production. An alternative highly preferred embodiment of the invention includes a method for inhibiting (e.g., reducing) IL-4 production. A highly preferred indication includes asthma. A highly preferred</p>

				<p>(including antibodies and agonists or antagonists of the invention) to mediate immunomodulation, stimulate immune cells, modulate immune cell polarization, and/or mediate humoral or cell-mediated immunity. Exemplary assays that test for immunomodulatory proteins evaluate the production of cytokines, such as IL-4, and the stimulation of immune cells, such as B cells, T cells, macrophages and mast cells. Such assays that may be used or routinely modified to test immunomodulatory activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Miraglia et al., J Biomolecular Screening 4:193-204 (1999); Rowland et al., "Lymphocytes: a practical approach" Chapter 6:138-160 (2000); Gonzalez et al., J Clin Lab Anal 8(5):277-283 (1994); Yssel et al., Res Immunol 144(8):610-616 (1993); Bagley et al., Nat Immunol 1(3):257-261 (2000); and van der Graaff et al., Rheumatology (Oxford)</p>	<p>indication includes allergy. A highly preferred indication includes rhinitis. Additional highly preferred indications include inflammation and inflammatory disorders. Highly preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, melanoma, and/or as described below under "Hyperproliferative Disorders"). Preferred indications include neoplasms and cancers, such as, for example, leukemia, lymphoma, melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Preferred indications include autoimmune diseases (e.g.,</p>
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75	HT3BF49	192	Production of IL-5	IL-5 FMAT. Assays for immunomodulatory proteins secreted by TH2 cells, mast cells, basophils, and eosinophils that stimulate eosinophil	38(3):214-220 (1999), the contents of each of which are herein incorporated by reference in its entirety. Human T cells that may be used according to these assays may be isolated using techniques disclosed herein or otherwise known in the art. Human T cells are primary human lymphocytes that mature in the thymus and express a T cell receptor and CD3, CD4, or CD8. These cells mediate humoral or cell-mediated immunity and may be preactivated to enhance responsiveness to immunomodulatory factors.	rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, and Lyme Disease. An additional preferred indication is infection (e.g., an infectious disease as described below under "Infectious Disease").
						A highly preferred embodiment of the invention includes a method for inhibiting (e.g., reducing) IL-5 production. An alternative highly preferred

			<p>function and B cell Ig production and promote polarization of CD4+ cells into TH2 cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to mediate immunomodulation, stimulate immune cell function, modulate B cell Ig production, modulate immune cell polarization, and/or mediate humoral or cell-mediated immunity. Exemplary assays that test for immunomodulatory proteins evaluate the production of cytokines, such as IL-5, and the stimulation of eosinophil function and B cell Ig production. Such assays that may be used or routinely modified to test immunomodulatory activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Miraglia et al., J Biomolecular Screening 4:193-204 (1999); Rowland et al.,</p>	<p>embodiment of the invention includes a method for stimulating (e.g., increasing) IL-5 production. A highly preferred embodiment of the invention includes a method for stimulating (e.g., increasing) immunoglobulin production. An alternative highly preferred embodiment of the invention includes a method for inhibiting (e.g., decreasing) immunoglobulin production. A highly preferred indication includes allergy. A highly preferred indication includes asthma. A highly preferred indication includes rhinitis. An additional highly preferred indication is infection (e.g., an infectious disease as described below under "Infectious Disease"), and inflammation and inflammatory disorders. Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic</p>
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				<p>"Lymphocytes: a practical approach" Chapter 6:138-160 (2000); Ohshima et al., Blood 92(9):3338-3345 (1998); Jung et al., Eur J Immunol 25(8):2413-2416 (1995); Mori et al., J Allergy Clin Immunol 106(1 Pt 2):558-564 (2000); and Koning et al., Cytokine 9(6):427-436 (1997), the contents of each of which are herein incorporated by reference in its entirety.</p> <p>Human T cells that may be used according to these assays may be isolated using techniques disclosed herein or otherwise known in the art. Human T cells are primary human lymphocytes that mature in the thymus and express a T cell receptor and CD3, CD4, or CD8. These cells mediate humoral or cell-mediated immunity and may be preactivated to enhance responsiveness to immunomodulatory factors.</p>	<p>lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, melanoma, and/or as described below under "Hyperproliferative Disorders"). Preferred indications include neoplasms and cancers, such as, leukemia, lymphoma, melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, leukemias, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, immune</p>
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					reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, and Lyme Disease.
76	HTEEW69	193	Proliferation of pre-adipose cells (such as 3T3-L1 cells)	Assays for the regulation (i.e. increases or decreases) of viability and proliferation of cells in vitro are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate viability and proliferation of pre-adipose cells and cell lines. For example, the CellTiter-Glo [®] Luminescent Cell Viability Assay (Promega Corp., Madison, WI, USA) can be used to measure the number of viable cells in culture based on quantitation of the ATP present which signals the presence of metabolically active cells. 3T3-L1 is a mouse preadipocyte cell line. It is a continuous substrain of 3T3 fibroblast cells developed through clonal isolation. Cells were differentiated to an adipose-like	

76	HTEEW69	193	Production of RANTES in endothelial cells (such as human umbilical vein endothelial cells (HUEVC))	<p>state before being used in the screen. See Green H and Meuth M., Cell 3: 127-133 (1974), which is herein incorporated by reference in its entirety.</p> <p>RANTES FMAT. Assays for immunomodulatory proteins that induce chemotaxis of T cells, monocytes, and eosinophils are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to mediate immunomodulation, induce chemotaxis, and/or mediate humoral or cell-mediated immunity. Exemplary assays that test for immunomodulatory proteins evaluate the production of cytokines, such as RANTES, and the induction of chemotactic responses in immune cells. Such assays that may be used or routinely modified to test immunomodulatory activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays</p>	
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76	HTEEW69	193	<p>Activation of transcription through cAMP response element in immune cells (such as T-</p>	<p>Assays for the activation of transcription through the cAMP response element are well-known in the art and may be used or routinely modified to</p>	<p>disclosed in Miraglia et al., J Biomolecular Screening 4:193-204 (1999); Rowland et al., "Lymphocytes: a practical approach" Chapter 6:138-160 (2000); Cocchi et al., Science 270(5243):1811-1815 (1995); and Robinson et al., Clin Exp Immunol 101(3):398-407 (1995), the contents of each of which are herein incorporated by reference in its entirety. Endothelial cells that may be used according to these assays are publicly available (e.g., through the ATCC). Exemplary endothelial cells that may be used according to these assays include human umbilical vein endothelial cells (HUVEC), which are endothelial cells which line venous blood vessels, and are involved in functions that include, but are not limited to, angiogenesis, vascular permeability, vascular tone, and immune cell extravasation.</p>	<p>Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or</p>
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			<p>cells).</p> <p>assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to increase cAMP, bind to CREB transcription factor, and modulate expression of genes involved in a wide variety of cell functions. Exemplary assays for transcription through the cAMP response element that may be used or routinely modified to test cAMP-response element activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); Black et al., Virus Genes 15(2):105-117 (1997); and Belkowski et al., J Immunol 161(2):659-665 (1998), the contents of each of which are herein incorporated by reference in its entirety. T cells that may be used according to these assays are publicly available (e.g., through the</p>	<p>"Cardiovascular Disorders"), and infection (e.g., an infectious disease as described below under "Infectious Disease"). Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response. Additional preferred indications include inflammation and inflammatory disorders. Highly preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Highly preferred indications include neoplasms and cancers, such as, leukemia, lymphoma (e.g., T cell lymphoma, Burkitt's lymphoma, non-Hodgkins lymphoma, Hodgkin's disease), melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other</p>
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				ATCC). Exemplary human T cells that may be used according to these assays include the JURKAT cell line, which is a suspension culture of leukemia cells that produce IL-2 when stimulated.	preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, and asthma and allergy.
76	HTEEW69	193	Activation of transcription through GAS response element in immune cells (such as T-cells).	Assays for the activation of transcription through the Gamma Interferon Activation Site (GAS) response element are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and	Highly preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Highly preferred indications include neoplasms and cancers, such as, for example, leukemia, lymphoma (e.g., T cell

				<p>agonists or antagonists of the invention) to regulate STAT transcription factors and modulate gene expression involved in a wide variety of cell functions. Exemplary assays for transcription through the GAS response element that may be used or routinely modified to test GAS-response element activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); Matikainen et al., Blood 93(6):1980-1991 (1999); and Henttinen et al., J Immunol 155(10):4582-4587 (1995), the contents of each of which are herein incorporated by reference in its entirety. Exemplary human T cells, such as the MOLT4 cell line, that may be used according to these assays are publicly available (e.g., through the ATCC).</p>	<p>lymphoma, Burkitt's lymphoma, non-Hodgkins lymphoma, Hodgkin's disease), melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response. Additional preferred indications include inflammation and inflammatory disorders. Highly preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"), and infection (e.g., viral</p>
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					infections, tuberculosis, infections associated with chronic granulomatous disease and malignant osteoporosis, and/or an infectious disease as described below under “Infectious Disease”). An additional preferred indication is idiopathic pulmonary fibrosis. Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, and asthma and allergy.
76	HTEEW69	193	Activation of transcription through NFKB response element in immune cells (such as T-cells).	Assays for the activation of transcription through the NFKB response element are well-known in the art and may be used or routinely modified to assess the ability of	Highly preferred indications include inflammation and inflammatory disorders. Highly preferred indications include blood disorders (e.g., as described below under “Immune

				<p>polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate NFκB transcription factors and modulate expression of immunomodulatory genes. Exemplary assays for transcription through the NFκB response element that may be used or routinely modified to test NFκB-response element activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); Black et al., Virus Gnes 15(2):105-117 (1997); and Fraser et al., 29(3):838-844 (1999), the contents of each of which are herein incorporated by reference in its entirety. Exemplary human T cells, such as the MOLT4, that may be used according to these assays are publicly available (e.g., through the ATCC).</p>	<p>Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Highly preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below), and immunodeficiencies (e.g., as described below). An additional highly preferred indication is infection (e.g., AIDS, and/or an infectious disease as described below under "Infectious Disease"). Highly preferred indications include neoplastic diseases (e.g., melanoma, leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Highly preferred indications include neoplasms and cancers, such as, for example, melanoma, renal cell carcinoma, leukemia, lymphoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example,</p>
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				hyperplasia, metaplasia, and/or dysplasia. Preferred indications also include anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, suppression of immune reactions to transplanted organs, asthma and allergy.
77	HTEHU59	194	Calcium flux in chondrocytes	<p>Assays for measuring calcium flux are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to mobilize calcium. Cells normally have very low concentrations of cytosolic calcium compared to much higher extracellular calcium. Extracellular factors can cause</p> <p>Preferred embodiments of the invention include using polypeptides of the invention (or antibodies, agonists, or antagonists thereof) in detection, diagnosis, prevention, and/or treatment of Bone and Cartilage Diseases, including but not limited to Arthritis, Cartilage repair, Bone Repair, Osteoporosis, and related tumors including chondrosarcomas,</p>

				<p>an influx of calcium, leading to activation of calcium responsive signaling pathways and alterations in cell functions. Exemplary assays that may be used or routinely modified to measure calcium flux in chondrocytes include assays disclosed in: Asada S, et al., <i>Inflamm Res</i>, 50(1):19-23 (2001); Schwartz Z, et al., <i>J Bone Miner Res</i>, 6(7):709-718 (1991); Iannotti JP, et al., <i>J Bone Joint Surg Am</i>, 67(1): 113-120 (1985); Sullivan E., et al., <i>Methods Mol Biol</i> 1999; 114:125-133 (1999), the contents of each of which is herein incorporated by reference in its entirety. Cells that may be used according to these assays are publicly available (e.g., through the ATCC) and/or may be routinely generated. Exemplary cells that may be used according to these assays include bovine chondrocytes.</p>	chondroblastomas, and chondromas.
77	HTEHU59	194	<p>Activation of transcription through GAS response element in immune cells (such as T-</p>	<p>Assays for the activation of transcription through the Gamma Interferon Activation Site (GAS) response element are well-known in the art and may</p>	<p>Highly preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, and/or as described below under “Hyperproliferative Disorders”).</p>

			cells).	<p>be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate STAT transcription factors and modulate gene expression involved in a wide variety of cell functions. Exemplary assays for transcription through the GAS response element that may be used or routinely modified to test GAS-response element activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); Matikainen et al., Blood 93(6):1980-1991 (1999); and Henttinen et al., J Immunol 155(10):4582-4587 (1995), the contents of each of which are herein incorporated by reference in its entirety. Exemplary human T cells, such as the</p>	<p>Highly preferred indications include neoplasms and cancers, such as, for example, leukemia, lymphoma (e.g., T cell lymphoma, Burkitt's lymphoma, non-Hodgkins lymphoma, Hodgkin's disease), melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response. Additional preferred indications include inflammation and inflammatory disorders. Highly preferred indications include blood disorders (e.g., as described below under "Immune</p>
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				<p>SUPT cell line, that may be used according to these assays are publicly available (e.g., through the ATCC).</p>	<p>Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"), and infection (e.g., viral infections, tuberculosis, infections associated with chronic granulomatous disease and malignant osteoporosis, and/or an infectious disease as described below under "Infectious Disease"). An additional preferred indication is idiopathic pulmonary fibrosis. Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, and asthma and allergy.</p>
77	HTEHU59	194	<p>Production of IL-10 and activation of T-</p>	<p>Assays for production of IL-10 and activation of T-cells are</p>	<p>Highly preferred indications include allergy and asthma.</p>

			<p>cells.</p> <p>well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-10 and/or activation of T-cells. Exemplary assays that may be used or routinely modified to assess the ability of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) to modulate IL-10 production and/or T-cell proliferation include, for example, assays such as disclosed and/or cited in: Robinson, DS, et al., "Th-2 cytokines in allergic disease" Br Med Bull; 56 (4): 956-968 (2000), and Cohn, et al., "T-helper type 2 cell-directed therapy for asthma" Pharmacology & Therapeutics; 88: 187-196 (2000); the contents of each of which are herein incorporated by reference in their entirety. Exemplary cells that may be used according to these assays include Th2 cells. IL10 secreted from Th2 cells</p>	<p>Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.</p>
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				<p>may be measured as a marker of Th2 cell activation. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6. Factors that induce differentiation and activation of Th2 cells play a major role in the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated via in vitro culture under Th2 polarizing conditions using peripheral blood lymphocytes isolated from cord blood.</p>	
78	HTEMQ17	195	<p>Regulation of apoptosis of immune cells (such as mast cells).</p>	<p>Caspase Apoptosis. Assays for caspase apoptosis are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate caspase protease-mediated apoptosis in immune cells (such as, for example, in mast cells). Mast cells are found in connective and mucosal tissues throughout the body, and their activation via immunoglobulin E-antigen, promoted by T helper cell type 2 cytokines, is an important</p>	<p>Preferred embodiments of the invention include using polypeptides of the invention (or antibodies, agonists, or antagonists thereof) in detection, diagnosis, prevention, and/or treatment of asthma, allergy, hypersensitivity and inflammation.</p>

				<p>component of allergic disease. Dysregulation of mast cell apoptosis may play a role in allergic disease and mast cell tumor survival. Exemplary assays for caspase apoptosis that may be used or routinely modified to test caspase apoptosis activity induced by polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in: Masuda A, et al., <i>J Biol Chem</i>, 276(28):26107-26113 (2001); Yeatman CF 2nd, et al., <i>J Exp Med</i>, 192(8):1093-1103 (2000); Lee et al., <i>FEBS Lett</i> 485(2-3): 122-126 (2000); Nor et al., <i>J Vasc Res</i> 37(3): 209-218 (2000); and Karsan and Harlan, <i>J Atheroscler Thromb</i> 3(2): 75-80 (1996); the contents of each of which are herein incorporated by reference in its entirety. Immune cells that may be used according to these assays are publicly available (e.g., through commercial sources). Exemplary immune cells that may be used according to these assays include mast cells such as the HMC human</p>
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79	HTGBK95	196	Production of IL-10 and activation of T-cells.	<p>mast cell line.</p> <p>Assays for production of IL-10 and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-10 and/or activation of T-cells.</p> <p>Exemplary assays that may be used or routinely modified to assess the ability of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) to modulate IL-10 production and/or T-cell proliferation include, for example, assays such as disclosed and/or cited in: Robinson, DS, et al., "Th-2 cytokines in allergic disease" Br Med Bull; 56 (4): 956-968 (2000), and Cohn, et al., "T-helper type 2 cell-directed therapy for asthma" Pharmacology & Therapeutics; 88: 187-196 (2000); the contents of each of which are herein incorporated by reference in</p>	<p>Highly preferred indications include allergy and asthma.</p> <p>Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.</p>
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				<p>their entirety. Exemplary cells that may be used according to these assays include Th2 cells. IL10 secreted from Th2 cells may be measured as a marker of Th2 cell activation. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6. Factors that induce differentiation and activation of Th2 cells play a major role in the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated via in vitro culture under Th2 polarizing conditions using peripheral blood lymphocytes isolated from cord blood.</p>	<p>Preferred embodiments of the invention include using polypeptides of the invention (or antibodies, agonists, or antagonists thereof) in detection, diagnosis, prevention, and/or treatment of asthma, allergy, hypersensitivity and inflammation.</p>
80	HTLEM16	197	<p>Regulation of apoptosis of immune cells (such as mast cells).</p>	<p>Caspase Apoptosis. Assays for caspase apoptosis are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate caspase protease-mediated apoptosis in immune cells (such as, for example, in mast cells). Mast cells are found in connective and mucosal tissues throughout</p>	

				<p>the body, and their activation via immunoglobulin E -antigen, promoted by T helper cell type 2 cytokines, is an important component of allergic disease. Dysregulation of mast cell apoptosis may play a role in allergic disease and mast cell tumor survival. Exemplary assays for caspase apoptosis that may be used or routinely modified to test caspase apoptosis activity induced by polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in: Masuda A, et al., <i>J Biol Chem</i>, 276(28):26107-26113 (2001); Yeatman CF 2nd, et al., <i>J Exp Med</i>, 192(8):1093-1103 (2000); Lee et al., <i>FEBS Lett</i> 485(2-3): 122-126 (2000); Nor et al., <i>J Vasc Res</i> 37(3): 209-218 (2000); and Karsan and Harlan, <i>J Atheroscler Thromb</i> 3(2): 75-80 (1996); the contents of each of which are herein incorporated by reference in its entirety. Immune cells that may be used according to these assays are publicly available (e.g., through commercial</p>
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				sources). Exemplary immune cells that may be used according to these assays include mast cells such as the HMC human mast cell line.	
80	HTLEM16	197	Activation of Endothelial Cell p38 or JNK Signaling Pathway.	<p>Kinase assay. JNK and p38 kinase assays for signal transduction that regulate cell proliferation, activation, or apoptosis are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to promote or inhibit cell proliferation, activation, and apoptosis. Exemplary assays for JNK and p38 kinase activity that may be used or routinely modified to test JNK and p38 kinase-induced activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Forrer et al., Biol Chem 379(8-9):1101-1110 (1998); Gupta et al., Exp Cell Res 247(2): 495-504 (1999); Kyriakis JM, Biochem Soc Symp 64:29-48 (1999); Chang</p> <p>A highly preferred embodiment of the invention includes a method for stimulating endothelial cell growth. An alternative highly preferred embodiment of the invention includes a method for inhibiting endothelial cell growth. A highly preferred embodiment of the invention includes a method for stimulating endothelial cell proliferation. An alternative highly preferred embodiment of the invention includes a method for inhibiting endothelial cell proliferation. A highly preferred embodiment of the invention includes a method for stimulating apoptosis of endothelial cells. An alternative highly preferred embodiment of the invention includes a method for inhibiting (e.g., decreasing) apoptosis of endothelial cells. A highly preferred embodiment of the invention includes a</p>	

			<p>and Karin, Nature 410(6824):37-40 (2001); and Cobb MH, Prog Biophys Mol Biol 71(3-4):479-500 (1999); the contents of each of which are herein incorporated by reference in its entirety. Endothelial cells that may be used according to these assays are publicly available (e.g., through the ATCC). Exemplary endothelial cells that may be used according to these assays include human umbilical vein endothelial cells (HÜVEC), which are endothelial cells which line venous blood vessels, and are involved in functions that include, but are not limited to, angiogenesis, vascular permeability, vascular tone, and immune cell extravasation.</p>	<p>method for stimulating (e.g., increasing) endothelial cell activation. An alternative highly preferred embodiment of the invention includes a method for inhibiting (e.g., decreasing) the activation of and/or inactivating endothelial cells. A highly preferred embodiment of the invention includes a method for stimulating angiogenesis. An alternative highly preferred embodiment of the invention includes a method for inhibiting angiogenesis. A highly preferred embodiment of the invention includes a method for reducing cardiac hypertrophy. An alternative highly preferred embodiment of the invention includes a method for inducing cardiac hypertrophy. Highly preferred indications include neoplastic diseases (e.g., as described below under “Hyperproliferative Disorders”), and disorders of the cardiovascular system (e.g., heart disease, congestive heart failure, hypertension, aortic stenosis, cardiomyopathy, valvular regurgitation, left ventricular dysfunction,</p>
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					<p>atherosclerosis and atherosclerotic vascular disease, diabetic nephropathy, intracardiac shunt, cardiac hypertrophy, myocardial infarction, chronic hemodynamic overload, and/or as described below under "Cardiovascular Disorders"). Highly preferred indications include cardiovascular, endothelial and/or angiogenic disorders (e.g., systemic disorders that affect vessels such as diabetes mellitus, as well as diseases of the vessels themselves, such as of the arteries, capillaries, veins and/or lymphatics). Highly preferred are indications that stimulate angiogenesis and/or cardiovascularization. Highly preferred are indications that inhibit angiogenesis and/or cardiovascularization. Highly preferred indications include antiangiogenic activity to treat solid tumors, leukemias, and Kaposi's sarcoma, and retinal disorders. Highly preferred indications include neoplasms and cancer, such as, Kaposi's sarcoma, hemangioma</p>
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					<p>(capillary and cavernous), glomus tumors, telangiectasia, bacillary angiomatosis, hemangioendothelioma, angiosarcoma, haemangiopericytoma, lymphangioma, lymphangiosarcoma. Highly preferred indications also include cancers such as, prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver, and urinary cancer. Preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Highly preferred indications also include arterial disease, such as, atherosclerosis, hypertension, coronary artery disease, inflammatory vasculitides, Reynaud's disease and Reynaud's phenomenon, aneurysms, restenosis; venous and lymphatic disorders such as thrombophlebitis, lymphangitis, and lymphedema; and other vascular disorders such as peripheral vascular disease, and cancer. Highly preferred indications also include trauma</p>
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				<p>such as wounds, burns, and injured tissue (e.g., vascular injury such as, injury resulting from balloon angioplasty, and atherosclerotic lesions), implant fixation, scarring, ischemia reperfusion injury, rheumatoid arthritis, cerebrovascular disease, renal diseases such as acute renal failure, and osteoporosis. Additional highly preferred indications include stroke, graft rejection, diabetic or other retinopathies, thrombotic and coagulative disorders, vasculitis, lymph angiogenesis, sexual disorders, age-related macular degeneration, and treatment /prevention of endometriosis and related conditions. Additional highly preferred indications include fibromas, heart disease, cardiac arrest, heart valve disease, and vascular disease. Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders").</p>

				<p>Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Additional preferred indications include inflammation and inflammatory disorders (such as acute and chronic inflammatory diseases, e.g., inflammatory bowel disease and Crohn's disease), and pain management.</p>
81	HTNBK13	198	<p>Regulation of viability and proliferation of pancreatic beta cells.</p>	<p>Assays for the regulation of viability and proliferation of cells in vitro are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate viability and proliferation of pancreatic beta cells. For example, the Cell Titer-Glo luminescent cell viability assay measures the number of viable cells in culture based on quantitation of the ATP present which signals the presence of metabolically active cells.</p> <p>A highly preferred indication is diabetes mellitus. An additional highly preferred indication is a complication associated with diabetes (e.g., diabetic retinopathy, diabetic nephropathy, kidney disease (e.g., renal failure, nephropathy and/or other diseases and disorders as described in the "Renal Disorders" section below), diabetic neuropathy, nerve disease and nerve damage (e.g., due to diabetic neuropathy), blood vessel blockage, heart disease, stroke, impotence (e.g., due to diabetic neuropathy or blood vessel</p>

				<p>Exemplary assays that may be used or routinely modified to test regulation of viability and proliferation of pancreatic beta cells by polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in: Friedrichsen BN, et al., Mol Endocrinol, 15(1):136-48 (2001); Huotari MA, et al., Endocrinology, 139(4):1494-9 (1998); Hugl SR, et al., J Biol Chem 1998 Jul 10;273(28):17771-9 (1998), the contents of each of which is herein incorporated by reference in its entirety. Pancreatic cells that may be used according to these assays are publicly available (e.g., through the ATCC) and/or may be routinely generated. Exemplary pancreatic cells that may be used according to these assays include rat INS-1 cells. INS-1 cells are a semi-adherent cell line established from cells isolated from an X-ray induced rat transplantable insulinoma. These cells retain characteristics typical of native pancreatic beta cells including glucose inducible</p>	<p>blockage), seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, endocrine disorders (as described in the "Endocrine Disorders" section below), neuropathy, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, and infection (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome and Dupuytren's contracture). An additional highly preferred indication is obesity and/or complications associated with obesity. Additional highly preferred indications include weight loss or alternatively, weight gain. Additional highly preferred</p>
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				insulin secretion. References: Asfari et al. Endocrinology 1992 130:167.	indications are complications associated with insulin resistance.
81	HTNBK13	198	Production of TNF alpha by dendritic cells	<p>TNFa FMAAT. Assays for immunomodulatory proteins produced by activated macrophages, T cells, fibroblasts, smooth muscle, and other cell types that exert a wide variety of inflammatory and cytotoxic effects on a variety of cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to mediate immunomodulation, modulate inflammation and cytotoxicity. Exemplary assays that test for immunomodulatory proteins evaluate the production of cytokines such as tumor necrosis factor alpha (TNFa), and the induction or inhibition of an inflammatory or cytotoxic response. Such assays that may be used or routinely modified to test immunomodulatory activity of polypeptides of the invention (including antibodies and</p>	<p>A highly preferred embodiment of the invention includes a method for inhibiting (e.g., decreasing) TNF alpha production. An alternative highly preferred embodiment of the invention includes a method for stimulating (e.g., increasing) TNF alpha production. Highly preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"), Highly preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response. Additional highly preferred indications include inflammation and</p>

			agonists or antagonists of the invention) include assays disclosed in Miraglia et al., J Biomolecular Screening 4:193-204(1999); Rowland et al., "Lymphocytes: a practical approach" Chapter 6:138-160 (2000); Verhasselt et al., Eur J Immunol 28(11):3886-3890 (1198); Dahlen et al., J Immunol 160(7):3585-3593 (1998); Verhasselt et al., J Immunol 158:2919-2925 (1997); and Nardelli et al., J Leukoc Biol 65:822-828 (1999), the contents of each of which are herein incorporated by reference in its entirety. Human dendritic cells that may be used according to these assays may be isolated using techniques disclosed herein or otherwise known in the art. Human dendritic cells are antigen presenting cells in suspension culture, which, when activated by antigen and/or cytokines, initiate and upregulate T cell proliferation and functional activities.	inflammatory disorders, and treating joint damage in patients with rheumatoid arthritis. An additional highly preferred indication is sepsis. Highly preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Additionally, highly preferred indications include neoplasms and cancers, such as, leukemia, lymphoma, melanoma, glioma (e.g., malignant glioma), solid tumors, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease,
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81	HTNBK13	198	Production of IL-10 and activation of T-cells.	Assays for production of IL-10 and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-10 and/or activation of T-cells. Exemplary assays that may be used or routinely modified to assess the ability of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) to modulate IL-10 production	inflammatory bowel disease, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, cardiac reperfusion injury, and asthma and allergy. An additional preferred indication is infection (e.g., an infectious disease as described below under "Infectious Disease").
				Assays for production of IL-10 and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-10 and/or activation of T-cells. Exemplary assays that may be used or routinely modified to assess the ability of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) to modulate IL-10 production	Highly preferred indications include allergy and asthma. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.

				<p>and/or T-cell proliferation include, for example, assays such as disclosed and/or cited in: Robinson, DS, et al., "Th-2 cytokines in allergic disease" Br Med Bull; 56 (4): 956-968 (2000), and Cohn, et al., "T-helper type 2 cell-directed therapy for asthma" Pharmacology & Therapeutics; 88: 187-196 (2000); the contents of each of which are herein incorporated by reference in their entirety. Exemplary cells that may be used according to these assays include Th2 cells. IL10 secreted from Th2 cells may be measured as a marker of Th2 cell activation. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6. Factors that induce differentiation and activation of Th2 cells play a major role in the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated via in vitro culture under Th2 polarizing conditions using peripheral blood lymphocytes isolated from cord blood.</p>				
	HTODN35	199	Production of IL-10	Assays for production of IL-10	Highly preferred indications			

82	and activation of T-cells.	and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-10 and/or activation of T-cells. Exemplary assays that may be used or routinely modified to assess the ability of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) to modulate IL-10 production and/or T-cell proliferation include, for example, assays such as disclosed and/or cited in: Robinson, DS, et al., "Th-2 cytokines in allergic disease" Br Med Bull; 56 (4): 956-968 (2000), and Cohn, et al., "T-helper type 2 cell-directed therapy for asthma" Pharmacology & Therapeutics; 88: 187-196 (2000); the contents of each of which are herein incorporated by reference in their entirety. Exemplary cells that may be used according to these assays include Th2 cells.	include allergy and asthma. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.
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				<p>IL10 secreted from Th2 cells may be measured as a marker of Th2 cell activation. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6. Factors that induce differentiation and activation of Th2 cells play a major role in the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated via in vitro culture under Th2 polarizing conditions using peripheral blood lymphocytes isolated from cord blood.</p>	
83	HTPDU17	200	Production of IL-4	<p>IL-4 FMA T. Assays for immunomodulatory proteins secreted by TH2 cells that stimulate B cells, T cells, macrophages and mast cells and promote polarization of CD4+ cells into TH2 cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to mediate immunomodulation, stimulate immune cells, modulate immune cell polarization, and/or mediate</p>	<p>A highly preferred embodiment of the invention includes a method for stimulating (e.g., increasing) IL-4 production. An alternative highly preferred embodiment of the invention includes a method for inhibiting (e.g., reducing) IL-4 production. A highly preferred indication includes asthma. A highly preferred indication includes allergy. A highly preferred indication includes rhinitis. Additional highly preferred indications include inflammation and inflammatory disorders.</p>

				<p>humoral or cell-mediated immunity. Exemplary assays that test for immunomodulatory proteins evaluate the production of cytokines, such as IL-4, and the stimulation of immune cells, such as B cells, T cells, macrophages and mast cells. Such assays that may be used or routinely modified to test immunomodulatory activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Miraglia et al., J Biomolecular Screening 4:193-204 (1999); Rowland et al., "Lymphocytes: a practical approach" Chapter 6:138-160 (2000); Gonzalez et al., J Clin Lab Anal 8(5):277-283 (1194); Yssel et al., Res Immunol 144(8):610-616 (1993); Bagley et al., Nat Immunol 1(3):257-261 (2000); and van der Graaff et al., Rheumatology (Oxford) 38(3):214-220 (1999), the contents of each of which are herein incorporated by reference in its entirety. Human T cells that may be used according to these assays may be isolated</p>	<p>Highly preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, melanoma, and/or as described below under "Hyperproliferative Disorders"). Preferred indications include neoplasms and cancers, such as, for example, leukemia, lymphoma, melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Preferred indications include</p>
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				<p>using techniques disclosed herein or otherwise known in the art. Human T cells are primary human lymphocytes that mature in the thymus and express a T cell receptor and CD3, CD4, or CD8. These cells mediate humoral or cell-mediated immunity and may be preactivated to enhance responsiveness to immunomodulatory factors.</p>	<p>anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, and Lyme Disease. An additional preferred indication is infection (e.g., an infectious disease as described below under "Infectious Disease").</p>
84	HTTDN24	201	<p>Activation of transcription through serum response element in immune cells (such as T-cells).</p>	<p>Assays for the activation of transcription through the Serum Response Element (SRE) are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate the serum response factors and modulate</p>	<p>A preferred embodiment of the invention includes a method for inhibiting (e.g., reducing) TNF alpha production. An alternative preferred embodiment of the invention includes a method for stimulating (e.g., increasing) TNF alpha production. Preferred indications include blood disorders (e.g., as</p>

			<p>the expression of genes involved in growth. Exemplary assays for transcription through the SRE that may be used or routinely modified to test SRE activity of the polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); and Black et al., Virus Genes 12(2):105-117 (1997), the content of each of which are herein incorporated by reference in its entirety. T cells that may be used according to these assays are publicly available (e.g., through the ATCC). Exemplary mouse T cells that may be used according to these assays include the CTLL cell line, which is an IL-2 dependent suspension culture of T cells with cytotoxic activity.</p>	<p>described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Highly preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response. Additional highly preferred indications include inflammation and inflammatory disorders, and treating joint damage in patients with rheumatoid arthritis. An additional highly preferred indication is sepsis. Highly preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Additionally, highly preferred indications include neoplasms and cancers, such as, for example, leukemia, lymphoma, melanoma, glioma (e.g.,</p>
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				<p>malignant glioma), solid tumors, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, cardiac reperfusion injury, and asthma and allergy. An additional preferred indication is infection (e.g., an infectious disease as described below under "Infectious Disease").</p>
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84	HTTDN24	201	<p>Production of IL-10 and activation of T-cells.</p>	<p>Assays for production of IL-10 and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-10 and/or activation of T-cells. Exemplary assays that may be used or routinely modified to assess the ability of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) to modulate IL-10 production and/or T-cell proliferation include, for example, assays such as disclosed and/or cited in: Robinson, DS, et al., "Th-2 cytokines in allergic disease" Br Med Bull; 56 (4): 956-968 (2000), and Cohn, et al., "T-helper type 2 cell-directed therapy for asthma" Pharmacology & Therapeutics; 88: 187-196 (2000); the contents of each of which are herein incorporated by reference in their entirety. Exemplary cells that may be used according to</p>	<p>Highly preferred indications include allergy and asthma. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.</p>
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85	HTTEE41	202	<p>Activation of transcription through serum response element in immune cells (such as T-cells).</p>	<p>these assays include Th2 cells. IL10 secreted from Th2 cells may be measured as a marker of Th2 cell activation. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6. Factors that induce differentiation and activation of Th2 cells play a major role in the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated via in vitro culture under Th2 polarizing conditions using peripheral blood lymphocytes isolated from cord blood.</p>	<p>A preferred embodiment of the invention includes a method for inhibiting (e.g., reducing) TNF alpha production. An alternative preferred embodiment of the invention includes a method for stimulating (e.g., increasing) TNF alpha production. Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"), Highly preferred indications</p>
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				<p>activity of the polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); and Black et al., Virus Genes 12(2):105-117 (1997), the content of each of which are herein incorporated by reference in its entirety. T cells that may be used according to these assays are publicly available (e.g., through the ATCC). Exemplary mouse T cells that may be used according to these assays include the CTLL cell line, which is an IL-2 dependent suspension culture of T cells with cytotoxic activity.</p>	<p>include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response. Additional highly preferred indications include inflammation and inflammatory disorders, and treating joint damage in patients with rheumatoid arthritis. An additional highly preferred indication is sepsis. Highly preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Additionally, highly preferred indications include neoplasms and cancers, such as, for example, leukemia, lymphoma, melanoma, glioma (e.g., malignant glioma), solid tumors, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other</p>
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					<p>preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, cardiac reperfusion injury, and asthma and allergy. An additional preferred indication is infection (e.g., an infectious disease as described below under "Infectious Disease").</p>
85	HTTEE4I	202	Stimulation of Calcium Flux in pancreatic beta cells.	Assays for measuring calcium flux are well-known in the art and may be used or routinely modified to assess the ability of	<p>A highly preferred indication is diabetes mellitus. An additional highly preferred indication is a complication</p>

				<p>polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to mobilize calcium. For example, the FLPR assay may be used to measure influx of calcium. Cells normally have very low concentrations of cytosolic calcium compared to much higher extracellular calcium. Extracellular factors can cause an influx of calcium, leading to activation of calcium responsive signaling pathways and alterations in cell functions. Exemplary assays that may be used or routinely modified to measure calcium flux by polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in: Satin LS, et al., Endocrinology, 136(10):4589-601 (1995); Mogami H, et al., Endocrinology, 136(7):2960-6 (1995); Richardson SB, et al., Biochem J, 288 (Pt 3):847-51 (1992); and, Meats, JE, et al., Cell Calcium 1989 Nov-Dec;10(8):535-41 (1989), the contents of each of which is herein incorporated by reference</p>	<p>associated with diabetes (e.g., diabetic retinopathy, diabetic nephropathy, kidney disease (e.g., renal failure, nephropathy and/or other diseases and disorders as described in the "Renal Disorders" section below), diabetic neuropathy, nerve disease and nerve damage (e.g., due to diabetic neuropathy), blood vessel blockage, heart disease, stroke, impotence (e.g., due to diabetic neuropathy or blood vessel blockage), seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, endocrine disorders (as described in the "Endocrine Disorders" section below), neuropathy, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, and infection (e.g.,</p>
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			<p>in its entirety. Pancreatic cells that may be used according to these assays are publicly available (e.g., through the ATCC) and/or may be routinely generated. Exemplary pancreatic cells that may be used according to these assays include HIT15 Cells. HIT15 are an adherent epithelial cell line established from Syrian hamster islet cells transformed with SV40. These cells express glucagon, somatostatin, and glucocorticoid receptors. The cells secrete insulin, which is stimulated by glucose and glucagon and suppressed by somatostatin or glucocorticoids. ATTC# CRL-1777 Refs: Lord and Ashcroft. Biochem. J. 219: 547-551; Santerre et al. Proc. Natl. Acad. Sci. USA 78: 4339-4343, 1981.</p>	<p>infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome and Dupuytren's contracture). An additional highly preferred indication is obesity and/or complications associated with obesity. Additional highly preferred indications include weight loss or alternatively, weight gain. Additional highly preferred indications are complications associated with insulin resistance.</p>
85	HTTEE41	202	<p>Production of IL-13 and activation of T-cells.</p>	<p>Highly preferred indications include allergy and asthma. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune</p>

				<p>invention) to stimulate or inhibit production of IL-13 and/or activation of T-cells. Exemplary assays for IL-13 production that may be used or routinely modified to test activity of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) include, for example, assays such as disclosed and/or cited in: Grunig, G, et al., "Requirement for IL-13 independently of IL-4 in Experimental asthma" Science; 282: 2261-2263 (1998), and Wills-Karp M, et al., "Interleukin-13: central mediator of allergic asthma" Science; 282: 2258-2261 (1998); the contents of each of which are herein incorporated by reference in their entirety. Exemplary cells that may be used according to these assays include Th2 cells. IL13, a Th2 type cytokine, is a potent stimulus for mucus production, airway hyper-responsiveness and allergic asthma. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6.</p>	<p>diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.</p>
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				<p>Factors that induce differentiation and activation of Th2 cells play a major role in the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated in vitro culture under Th2 polarizing conditions using peripheral blood lymphocytes isolated from cord blood.</p>	
86	HTXJD85	203	<p>Production of IL-10 and activation of T-cells.</p>	<p>Assays for production of IL-10 and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-10 and/or activation of T-cells. Exemplary assays that may be used or routinely modified to assess the ability of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) to modulate IL-10 production and/or T-cell proliferation include, for example, assays such as disclosed and/or cited in: Robinson, DS, et al., "Th-2</p>	<p>Highly preferred indications include allergy and asthma. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.</p>

				<p>cytokines in allergic disease" Br Med Bull; 56 (4): 956-968 (2000), and Cohn, et al., "T-helper type 2 cell-directed therapy for asthma" Pharmacology & Therapeutics; 88: 187-196 (2000); the contents of each of which are herein incorporated by reference in their entirety. Exemplary cells that may be used according to these assays include Th2 cells. IL10 secreted from Th2 cells may be measured as a marker of Th2 cell activation. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6. Factors that induce differentiation and activation of Th2 cells play a major role in the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated via in vitro culture under Th2 polarizing conditions using peripheral blood lymphocytes isolated from cord blood.</p>	
				<p>Assays for the regulation of viability and proliferation of cells in vitro are well-known in the art and may be used or routinely modified to assess the</p>	<p>A highly preferred indication is diabetes mellitus. An additional highly preferred indication is a complication associated with diabetes (e.g., diabetic</p>
87	HUVDJ48	204	Regulation of viability and proliferation of pancreatic beta cells.		

			<p>ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate viability and proliferation of pancreatic beta cells. For example, the Cell Titer-Glo luminescent cell viability assay measures the number of viable cells in culture based on quantitation of the ATP present which signals the presence of metabolically active cells. Exemplary assays that may be used or routinely modified to test regulation of viability and proliferation of pancreatic beta cells by polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in: Friedrichsen BN, et al., Mol Endocrinol, 15(1):136-48 (2001); Huotari MA, et al., Endocrinology, 139(4):1494-9 (1998); Hugl SR, et al., J Biol Chem 1998 Jul 10;273(28):17771-9 (1998), the contents of each of which is herein incorporated by reference in its entirety. Pancreatic cells that may be used according to these assays are publicly</p>	<p>retinopathy, diabetic nephropathy, kidney disease (e.g., renal failure, nephropathy and/or other diseases and disorders as described in the "Renal Disorders" section below), diabetic neuropathy, nerve disease and nerve damage (e.g., due to diabetic neuropathy), blood vessel blockage, heart disease, stroke, impotence (e.g., due to diabetic neuropathy or blood vessel blockage), seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, endocrine disorders (as described in the "Endocrine Disorders" section below), neuropathy, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, and infection (e.g., infectious diseases and disorders</p>
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			<p>available (e.g., through the ATCC) and/or may be routinely generated. Exemplary pancreatic cells that may be used according to these assays include rat INS-1 cells. INS-1 cells are a semi-adherent cell line established from cells isolated from an X-ray induced rat transplantable insulinoma. These cells retain characteristics typical of native pancreatic beta cells including glucose inducible insulin secretion. References: Asfari et al. Endocrinology 1992 130:167.</p>	<p>as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome and Dupuytren's contracture). An additional highly preferred indication is obesity and/or complications associated with obesity. Additional highly preferred indications include weight loss or alternatively, weight gain. Additional highly preferred indications are complications associated with insulin resistance.</p>
87	HUVDJ48	204	<p>Activation of JNK Signaling Pathway in immune cells (such as eosinophils).</p>	<p>Highly preferred indications include asthma, allergy, hypersensitivity reactions, inflammation, and inflammatory disorders. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as</p>

				<p>of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Forrer et al., Biol Chem 379(8-9):1101-1110 (1998); Gupta et al., Exp Cell Res 247(2): 495-504 (1999); Kyriakis JM, Biochem Soc Symp 64:29-48 (1999); Chang and Karin, Nature 410(6824):37-40 (2001); and Cobb MH, Prog Biophys Mol Biol 71(3-4):479-500 (1999); the contents of each of which are herein incorporated by reference in its entirety. Exemplary cells that may be used according to these assays include eosinophils. Eosinophils are important in the late stage of allergic reactions; they are recruited to tissues and mediate the inflammatory response of late stage allergic reaction. Moreover, exemplary assays that may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to modulate signal transduction, cell</p>	<p>described below). Highly preferred indications also include boosting or inhibiting immune cell proliferation. Preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Highly preferred indications include boosting an eosinophil-mediated immune response, and suppressing an eosinophil-mediated immune response.</p>
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				proliferation, activation, or apoptosis in eosinophils include assays disclosed and/or cited in: Zhang JP, et al., "Role of caspases in dexamethasone-induced apoptosis and activation of c-Jun NH2-terminal kinase and p38 mitogen-activated protein kinase in human eosinophils" Clin Exp Immunol; Oct;122(1):20-7 (2000); Hebestreit H, et al., "Disruption of fas receptor signaling by nitric oxide in eosinophils" J Exp Med; Feb 2;187(3):415-25 (1998); J Allergy Clin Immunol 1999 Sep;104(3 Pt 1):565-74; and, Sousa AR, et al., "In vivo resistance to corticosteroids in bronchial asthma is associated with enhanced phosphorylation of JUN N-terminal kinase and failure of prednisolone to inhibit JUN N-terminal kinase phosphorylation" J Allergy Clin Immunol; Sep;104(3 Pt 1):565-74 (1999); the contents of each of which are herein incorporated by reference in its entirety.	
88	HWBBU75	205	Activation of JNK Signaling Pathway in immune cells	Kinase assay. JNK kinase assays for signal transduction that regulate cell proliferation, Highly preferred indications include asthma, allergy, hypersensitivity reactions,	

			<p>(such as eosinophils).</p>	<p>activation, or apoptosis are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to promote or inhibit cell proliferation, activation, and apoptosis. Exemplary assays for JNK kinase activity that may be used or routinely modified to test JNK kinase-induced activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Forrer et al., Biol Chem 379(8-9):1101-1110 (1998); Gupta et al., Exp Cell Res 247(2): 495-504 (1999); Kyriakis JM, Biochem Soc Symp 64:29-48 (1999); Chang and Karin, Nature 410(6824):37-40 (2001); and Cobb MH, Prog Biophys Mol Biol 71(3-4):479-500 (1999); the contents of each of which are herein incorporated by reference in its entirety. Exemplary cells that may be used according to these assays include eosinophils.</p>	<p>inflammation, and inflammatory disorders. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below). Highly preferred indications also include boosting or inhibiting immune cell proliferation. Preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Highly preferred indications include boosting an eosinophil-mediated immune response, and suppressing an eosinophil-mediated immune response.</p>
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				<p>Eosinophils are important in the late stage of allergic reactions; they are recruited to tissues and mediate the inflammatory response of late stage allergic reaction. Moreover, exemplary assays that may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to modulate signal transduction, cell proliferation, activation, or apoptosis in eosinophils include assays disclosed and/or cited in: Zhang JP, et al., "Role of caspases in dexamethasone-induced apoptosis and activation of c-Jun NH2-terminal kinase and p38 mitogen-activated protein kinase in human eosinophils" Clin Exp Immunol; Oct;122(1):20-7 (2000); Hebestreit H, et al., "Disruption of fas receptor signaling by nitric oxide in eosinophils" J Exp Med; Feb 2;187(3):415-25 (1998); J Allergy Clin Immunol 1999 Sep;104(3 Pt 1):565-74; and, Sousa AR, et al., "In vivo resistance to corticosteroids in bronchial asthma is associated</p>
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89	HWHPB78	206	<p>Activation of transcription through NFKB response element in immune cells (such as natural killer cells).</p>	<p>with enhanced phosphorylation of JUN N-terminal kinase and failure of prednisolone to inhibit JUN N-terminal kinase phosphorylation" J Allergy Clin Immunol; Sep;104(3 Pt 1):565-74 (1999); the contents of each of which are herein incorporated by reference in its entirety.</p> <p>Assays for the activation of transcription through the NFKB response element are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate NFKB transcription factors and modulate expression of immunomodulatory genes. Exemplary assays for transcription through the NFKB response element that may be used or routinely modified to test NFKB-response element activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene</p>	<p>Highly preferred indications include inflammation and inflammatory disorders. Highly preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Highly preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below), and immunodeficiencies (e.g., as described below). An additional highly preferred indication is infection (e.g., AIDS, and/or an infectious disease as described below under "Infectious Disease"). Highly preferred indications include neoplastic</p>
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				<p>66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); Valle Blazquez et al, Immunology 90(3):455-460 (1997); Aramburau et al., J Exp Med 82(3):801-810 (1995); and Fraser et al., 29(3):838-844 (1999), the contents of each of which are herein incorporated by reference in its entirety. NK cells that may be used according to these assays are publicly available (e.g., through the ATCC). Exemplary human NK cells that may be used according to these assays include the NKL cell line, which is a human natural killer cell line established from the peripheral blood of a patient with large granular lymphocytic leukemia. This IL-2 dependent suspension culture cell line has a morphology resembling that of activated NK cells.</p>	<p>diseases (e.g., melanoma, leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Highly preferred indications include neoplasms and cancers, such as, for example, melanoma, renal cell carcinoma, leukemia, lymphoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications also include anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, suppression of immune</p>
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90	HWLBO67	207	Production of IL-10 and activation of T-cells.	Assays for production of IL-10 and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-10 and/or activation of T-cells. Exemplary assays that may be used or routinely modified to assess the ability of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) to modulate IL-10 production and/or T-cell proliferation include, for example, assays such as disclosed and/or cited in: Robinson, DS, et al., "Th-2 cytokines in allergic disease" Br Med Bull; 56 (4): 956-968 (2000), and Cohn, et al., "T-helper type 2 cell-directed therapy for asthma" Pharmacology & Therapeutics; 88: 187-196 (2000); the contents of each of which are herein	reactions to transplanted organs, asthma and allergy. Highly preferred indications include allergy and asthma. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.
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				<p>incorporated by reference in their entirety. Exemplary cells that may be used according to these assays include Th2 cells. IL10 secreted from Th2 cells may be measured as a marker of Th2 cell activation. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6. Factors that induce differentiation and activation of Th2 cells play a major role in the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated via in vitro culture under Th2 polarizing conditions using peripheral blood lymphocytes isolated from cord blood.</p>	
91	HWLGP26	208	<p>Production of IL-10 and activation of T-cells.</p>	<p>Assays for production of IL-10 and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-10 and/or activation of T-cells. Exemplary assays that may be used or routinely modified to</p>	<p>Highly preferred indications include allergy and asthma. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below),</p>

				<p>assess the ability of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) to modulate IL-10 production and/or T-cell proliferation include, for example, assays such as disclosed and/or cited in: Robinson, DS, et al., "Th-2 cytokines in allergic disease" Br Med Bull; 56 (4): 956-968 (2000), and Cohn, et al., "T-helper type 2 cell-directed therapy for asthma" Pharmacology & Therapeutics; 88: 187-196 (2000); the contents of each of which are herein incorporated by reference in their entirety. Exemplary cells that may be used according to these assays include Th2 cells. IL10 secreted from Th2 cells may be measured as a marker of Th2 cell activation. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6. Factors that induce differentiation and activation of Th2 cells play a major role in the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated via in vitro culture under Th2</p>	<p>immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.</p>
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92	HILCA24	209	Regulation of viability and proliferation of pancreatic beta cells.	polarizing conditions using peripheral blood lymphocytes isolated from cord blood. Assays for the regulation of viability and proliferation of cells in vitro are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate viability and proliferation of pancreatic beta cells. For example, the Cell Titer-Glo luminescent cell viability assay measures the number of viable cells in culture based on quantitation of the ATP present which signals the presence of metabolically active cells. Exemplary assays that may be used or routinely modified to test regulation of viability and proliferation of pancreatic beta cells by polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in: Friedrichsen BN, et al., Mol Endocrinol, 15(1):136-48 (2001); Huotari MA, et al.,	A highly preferred indication is diabetes mellitus. An additional highly preferred indication is a complication associated with diabetes (e.g., diabetic retinopathy, diabetic nephropathy, kidney disease (e.g., renal failure, nephropathy and/or other diseases and disorders as described in the "Renal Disorders" section below), diabetic neuropathy, nerve disease and nerve damage (e.g., due to diabetic neuropathy), blood vessel blockage, heart disease, stroke, impotence (e.g., due to diabetic neuropathy or blood vessel blockage), seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyposmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below),
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				<p>Endocrinology, 139(4):1494-9 (1998); Hugl SR, et al., J Biol Chem 1998 Jul 10;273(28):17771-9 (1998), the contents of each of which is herein incorporated by reference in its entirety. Pancreatic cells that may be used according to these assays are publicly available (e.g., through the ATCC) and/or may be routinely generated. Exemplary pancreatic cells that may be used according to these assays include rat INS-1 cells. INS-1 cells are a semi-adherent cell line established from cells isolated from an X-ray induced rat transplantable insulinoma. These cells retain characteristics typical of native pancreatic beta cells including glucose inducible insulin secretion. References: Asfari et al. Endocrinology 1992 130:167.</p>	<p>dyslipidemia, endocrine disorders (as described in the "Endocrine Disorders" section below), neuropathy, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, and infection (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome and Dupuytren's contracture). An additional highly preferred indication is obesity and/or complications associated with obesity. Additional highly preferred indications include weight loss or alternatively, weight gain. Additional highly preferred indications are complications associated with insulin resistance.</p>
92	HILCA24	209	<p>Activation of transcription through STAT6 response element in immune cells (such as T-cells).</p>	<p>Assays for the activation of transcription through the Signal Transducers and Activators of Transcription (STAT6) response element are well-known in the art and may be used or routinely modified to assess the ability of</p>	<p>A highly preferred indication is allergy. Another highly preferred indication is asthma. Additional highly preferred indications include inflammation and inflammatory</p>

				<p>polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate STAT6 transcription factors and modulate the expression of multiple genes. Exemplary assays for transcription through the STAT6 response element that may be used or routinely modified to test STAT6 response element activity of the polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); Georas et al., Blood 92(12):4529-4538 (1998); Moffatt et al., Transplantation 69(7):1521-1523 (2000); Curiel et al., Eur J Immunol 27(8):1982-1987 (1997); and Masuda et al., J Biol Chem 275(38):29331-29337 (2000), the contents of each of which are herein incorporated by reference in its entirety. T cells that may be used according</p>	<p>disorders. Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, melanoma, and/or as described below under "Hyperproliferative Disorders"). Preferred indications include neoplasms and cancers, such as, leukemia, lymphoma, melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include anemia,</p>
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				to these assays are publicly available (e.g., through the ATCC). Exemplary T cells that may be used according to these assays include the HT2 cell line, which is an IL-2 dependent suspension culture of T cells that also respond to IL-4.	pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, and Lyme Disease. An additional preferred indication is infection (e.g., an infectious disease as described below under "Infectious Disease").
92	HILCA24	209	Activation of transcription through STAT6 response element in immune cells (such as T-cells).	Assays for the activation of transcription through the Signal Transducers and Activators of Transcription (STAT6) response element are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate STAT6	<p>A highly preferred indication is allergy. Another highly preferred indication is asthma. Additional highly preferred indications include inflammation and inflammatory disorders. Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related</p>

			<p>transcription factors and modulate the expression of multiple genes. Exemplary assays for transcription through the STAT6 response element that may be used or routinely modified to test STAT6 response element activity of the polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); Georas et al., Blood 92(12):4529-4538 (1998); Moffatt et al., Transplantation 69(7):1521-1523 (2000); Curiel et al., Eur J Immunol 27(8):1982-1987 (1997); and Masuda et al., J Biol Chem 275(38):29331-29337 (2000), the contents of each of which are herein incorporated by reference in its entirety. T cells that may be used according to these assays are publicly available (e.g., through the ATCC). Exemplary T cells that may be used according to these</p>	<p>Disorders", and/or "Cardiovascular Disorders"). Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, melanoma, and/or as described below under "Hyperproliferative Disorders"). Preferred indications include neoplasms and cancers, such as, leukemia, lymphoma, melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple</p>
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				assays include the SUPT cell line, which is a suspension culture of IL-2 and IL-4 responsive T cells.	myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, and Lyme Disease. An additional preferred indication is infection (e.g., an infectious disease as described below under "Infectious Disease").
93	HE2CA60	210	Activation of transcription through serum response element in immune cells (such as T-cells).	Assays for the activation of transcription through the Serum Response Element (SRE) are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate the serum response factors and modulate the expression of genes involved in growth. Exemplary assays for transcription through the SRE that may be used or routinely modified to test SRE	A preferred embodiment of the invention includes a method for inhibiting (e.g., reducing) TNF alpha production. An alternative preferred embodiment of the invention includes a method for stimulating (e.g., increasing) TNF alpha production. Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"), Highly preferred indications

				<p>activity of the polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); and Black et al., Virus Genes 12(2):105-117 (1997), the content of each of which are herein incorporated by reference in its entirety. T cells that may be used according to these assays are publicly available (e.g., through the ATCC). Exemplary mouse T cells that may be used according to these assays include the CTLL cell line, which is an IL-2 dependent suspension culture of T cells with cytotoxic activity.</p>	<p>include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response. Additional highly preferred indications include inflammation and inflammatory disorders, and treating joint damage in patients with rheumatoid arthritis. An additional highly preferred indication is sepsis. Highly preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Additionally, highly preferred indications include neoplasms and cancers, such as, for example, leukemia, lymphoma, melanoma, glioma (e.g., malignant glioma), solid tumors, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other</p>
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					<p>preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, cardiac reperfusion injury, and asthma and allergy. An additional preferred indication is infection (e.g., an infectious disease as described below under "Infectious Disease").</p> <p>A highly preferred embodiment of the invention includes a method for stimulating (e.g., increasing)</p>
93	HE2CA60	210	Production of IL-4	IL-4 FMAT. Assays for immunomodulatory proteins secreted by TH2 cells that stimulate B cells, T cells,	

			<p>macrophages and mast cells and promote polarization of CD4+ cells into TH2 cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to mediate immunomodulation, stimulate immune cells, modulate immune cell polarization, and/or mediate humoral or cell-mediated immunity. Exemplary assays that test for immunomodulatory proteins evaluate the production of cytokines, such as IL-4, and the stimulation of immune cells, such as B cells, T cells, macrophages and mast cells. Such assays that may be used or routinely modified to test immunomodulatory activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Miraglia et al., J Biomolecular Screening 4:193-204 (1999); Rowland et al., "Lymphocytes: a practical approach" Chapter 6:138-160</p>	<p>IL-4 production. An alternative highly preferred embodiment of the invention includes a method for inhibiting (e.g., reducing) IL-4 production. A highly preferred indication includes asthma. A highly preferred indication includes allergy. A highly preferred indication includes rhinitis. Additional highly preferred indications include inflammation and inflammatory disorders. Highly preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, melanoma, and/or as described below under "Hyperproliferative Disorders"). Preferred indications include neoplasms and cancers, such as, for example, leukemia, lymphoma, melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include blood</p>
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				<p>(2000); Gonzalez et al., J Clin Lab Anal 8(5):277-283 (1194); Yssel et al., Res Immunol 144(8):610-616 (1993); Bagley et al., Nat Immunol 1(3):257-261 (2000); and van der Graaff et al., Rheumatology (Oxford) 38(3):214-220 (1999), the contents of each of which are herein incorporated by reference in its entirety. Human T cells that may be used according to these assays may be isolated using techniques disclosed herein or otherwise known in the art. Human T cells are primary human lymphocytes that mature in the thymus and express a T cell receptor and CD3, CD4, or CD8. These cells mediate humoral or cell-mediated immunity and may be preactivated to enhance responsiveness to immunomodulatory factors.</p>	<p>disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, and Lyme Disease. An additional preferred indication is infection (e.g., an infectious disease as described below under "Infectious</p>
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93	HE2CA60	210	Production of VCAM in endothelial cells (such as human umbilical vein endothelial cells (HUEVC))	Assays for measuring expression of VCAM are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate VCAM expression. For example, FRET may be used to measure the upregulation of cell surface VCAM-1 expression in endothelial cells. Endothelial cells are cells that line blood vessels, and are involved in functions that include, but are not limited to, angiogenesis, vascular permeability, vascular tone, and immune cell extravasation. Exemplary endothelial cells that may be used according to these assays include human umbilical vein endothelial cells (HUEVC), which are available from commercial sources. The expression of VCAM (CD106), a membrane-associated protein, can be upregulated by cytokines or other factors, and contributes	Disease"). Highly preferred indications include inflammation (acute and chronic), restnosis, atherosclerosis, asthma and allergy. Highly preferred indications include inflammation and inflammatory disorders, immunological disorders, neoplastic disorders (e.g. cancer/tumorigenesis), and cardiovascular disorders (such as described below under "Immune Activity", "Blood-Related Disorders", "Hyperproliferative Disorders" and/or "Cardiovascular Disorders"). Highly preferred indications include neoplasms and cancers such as, for example, leukemia, lymphoma, melanoma, renal cell carcinoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia.
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				to the extravasation of lymphocytes, leucocytes and other immune cells from blood vessels; thus VCAM expression plays a role in promoting immune and inflammatory responses.	
94	HPWTF23	211	Production of IL-13 and activation of T-cells.	Assays for production of IL-13 and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-13 and/or activation of T-cells. Exemplary assays for IL-13 production that may be used or routinely modified to test activity of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) include, for example, assays such as disclosed and/or cited in: Grunig, G, et al., "Requirement for IL-13 independently of IL-4 in Experimental asthma" Science;282: 2261-2263 (1998),	Highly preferred indications include allergy and asthma. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.

95	HGCAC19	212	Activation of JNK Signaling Pathway in immune cells (such as eosinophils).	<p>and Wills-Karp M, et al., "Interleukin-13: central mediator of allergic asthma" Science; 282: 2258-2261 (1998); the contents of each of which are herein incorporated by reference in their entirety. Exemplary cells that may be used according to these assays include Th2 cells. IL13, a Th2 type cytokine, is a potent stimulus for mucus production, airway hyper-responsiveness and allergic asthma. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6. Factors that induce differentiation and activation of Th2 cells play a major role in the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated in vitro culture under Th2 polarizing conditions using peripheral blood lymphocytes isolated from cord blood.</p> <p>Kinase assay. JNK kinase assays for signal transduction that regulate cell proliferation, activation, or apoptosis are well known in the art and may be used or routinely modified to</p>	<p>Highly preferred indications include asthma, allergy, hypersensitivity reactions, inflammation, and inflammatory disorders. Additional highly preferred indications include</p>
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				<p>assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to promote or inhibit cell proliferation, activation, and apoptosis. Exemplary assays for JNK kinase activity that may be used or routinely modified to test JNK kinase-induced activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Forrer et al., Biol Chem 379(8-9):1101-1110 (1998); Gupta et al., Exp Cell Res 247(2): 495-504 (1999); Kyriakis JM, Biochem Soc Symp 64:29-48 (1999); Chang and Karin, Nature 410(6824):37-40 (2001); and Cobb MH, Prog Biophys Mol Biol 71(3-4):479-500 (1999); the contents of each of which are herein incorporated by reference in its entirety. Exemplary cells that may be used according to these assays include eosinophils. Eosinophils are important in the late stage of allergic reactions; they are recruited to tissues and</p>	<p>immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below). Highly preferred indications also include boosting or inhibiting immune cell proliferation. Preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, and/or as described below under "Hyperproliferative Disorders"). Highly preferred indications include boosting an eosinophil-mediated immune response, and suppressing an eosinophil-mediated immune response.</p>
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				<p>mediate the inflammatory response of late stage allergic reaction. Moreover, exemplary assays that may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to modulate signal transduction, cell proliferation, activation, or apoptosis in eosinophils include assays disclosed and/or cited in: Zhang JP, et al., "Role of caspases in dexamethasone-induced apoptosis and activation of c-Jun NH2-terminal kinase and p38 mitogen-activated protein kinase in human eosinophils" Clin Exp Immunol; Oct;122(1):20-7 (2000); Hebestreit H, et al., "Disruption of fas receptor signaling by nitric oxide in eosinophils" J Exp Med; Feb 2;187(3):415-25 (1998); J Allergy Clin Immunol 1999 Sep;104(3 Pt 1):565-74; and, Sousa AR, et al., "In vivo resistance to corticosteroids in bronchial asthma is associated with enhanced phosphorylation of JUN N-terminal kinase and failure of prednisolone to inhibit</p>
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95	HGCAC19	212	<p>Activation of transcription through GATA-3 response element in immune cells (such as mast cells).</p>	<p>JUN N-terminal kinase phosphorylation" J Allergy Clin Immunol; Sep;104(3 Pt 1):565-74 (1999); the contents of each of which are herein incorporated by reference in its entirety.</p> <p>This reporter assay measures activation of the GATA-3 signaling pathway in HMC-1 human mast cell line. Activation of GATA-3 in mast cells has been linked to cytokine and chemokine production. Assays for the activation of transcription through the GATA3 response element are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate GATA3 transcription factors and modulate expression of mast cell genes important for immune response development. Exemplary assays for transcription through the GATA3 response element that may be used or routinely modified to test GATA3-</p>	<p>Highly preferred indications include allergy, asthma, and rhinitis. Additional preferred indications include infection (e.g., an infectious disease as described below under "Infectious Disease"), and inflammation and inflammatory disorders. Preferred indications also include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, melanoma, prostate, breast, lung, colon, pancreatic,</p>
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			<p>response element activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); Flavell et al., Cold Spring Harb Symp Quant Biol 64:563-571 (1999); Rodriguez-Palmero et al., Eur J Immunol 29(12):3914-3924 (1999); Zheng and Flavell, Cell 89(4):587-596 (1997); and Henderson et al., Mol Cell Biol 14(6):4286-4294 (1994), the contents of each of which are herein incorporated by reference in its entirety. Mast cells that may be used according to these assays are publicly available (e.g., through the ATCC). Exemplary human mast cells that may be used according to these assays include the HMC-1 cell line, which is an immature human mast cell line established from the peripheral blood of a patient with mast cell leukemia, and exhibits many</p>	<p>esophageal, stomach, brain, liver, and urinary tract cancers and/or as described below under "Hyperproliferative Disorders"). Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, leukemias, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, and Lyme Disease.</p>
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95	HGCAC19	212	<p>Activation of transcription through NFAT response element in immune cells (such as mast cells).</p>	<p>characteristics of immature mast cells.</p> <p>This reporter assay measures activation of the NFAT signaling pathway in HMC-1 human mast cell line. Activation of NFAT in mast cells has been linked to cytokine and chemokine production. Assays for the activation of transcription through the Nuclear Factor of Activated T cells (NFAT) response element are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate NFAT transcription factors and modulate expression of genes involved in immunomodulatory functions. Exemplary assays for transcription through the NFAT response element that may be used or routinely modified to test NFAT-response element activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays</p>	<p>Highly preferred indications include allergy, asthma, and rhinitis. Additional preferred indications include infection (e.g., an infectious disease as described below under "Infectious Disease"), and inflammation and inflammatory disorders. Preferred indications also include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Preferred indications include neoplastic diseases (e.g., leukemia, lymphoma, melanoma, prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver, and urinary tract cancers and/or as described below under "Hyperproliferative Disorders").</p>
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				disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); De Boer et al., Int J Biochem Cell Biol 31(10):1221-1236 (1999); Ali et al., J Immunol 165(12):7215-7223 (2000); Hutchinson and McCloskey, J Biol Chem 270(27):16333-16338 (1995), and Turner et al., J Exp Med 188:527-537 (1998), the contents of each of which are herein incorporated by reference in its entirety. Mast cells that may be used according to these assays are publicly available (e.g., through the ATCC). Exemplary human mast cells that may be used according to these assays include the HMC-1 cell line, which is an immature human mast cell line established from the peripheral blood of a patient with mast cell leukemia, and exhibits many characteristics of immature mast cells.	Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Preferred indications include anemia, pancytopenia, leukopenia, thrombocytopenia, leukemias, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, AIDS, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, suppression of immune reactions to transplanted organs and tissues, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, and Lyme Disease.
95	HGCAC19	212	Activation of transcription through	Assays for the activation of transcription through the CD28	A highly preferred embodiment of the invention

			<p>CD28 response element in immune cells (such as T-cells).</p>	<p>response element are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate IL-2 expression in T cells. Exemplary assays for transcription through the CD28 response element that may be used or routinely modified to test CD28-response element activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); McGuire and Iacobelli, J Immunol 159(3):1319-1327 (1997); Parra et al., J Immunol 166(4):2437-2443 (2001); and Butscher et al., J Biol Chem 3(1):552-560 (1998), the contents of each of which are herein incorporated by reference in its entirety. T cells that may be used according</p>	<p>includes a method for stimulating T cell proliferation. An alternative highly preferred embodiment of the invention includes a method for inhibiting T cell proliferation. A highly preferred embodiment of the invention includes a method for activating T cells. An alternative highly preferred embodiment of the invention includes a method for inhibiting the activation of and/or inactivating T cells. A highly preferred embodiment of the invention includes a method for stimulating (e.g., increasing) IL-2 production. An alternative highly preferred embodiment of the invention includes a method for inhibiting (e.g., reducing) IL-2 production. Additional highly preferred indications include inflammation and inflammatory disorders. Highly preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response,</p>
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				<p>to these assays are publicly available (e.g., through the ATCC). Exemplary human T cells that may be used according to these assays include the JURKAT cell line, which is a suspension culture of leukemia cells that produce IL-2 when stimulated.</p>	<p>and suppressing a T cell-mediated immune response. An additional highly preferred indication includes infection (e.g., AIDS, and/or as described below under “Infectious Disease”). Highly preferred indications include neoplastic diseases (e.g., melanoma, renal cell carcinoma, leukemia, lymphoma, and/or as described below under “Hyperproliferative Disorders”). Highly preferred indications include neoplasms and cancers, such as, for example, melanoma (e.g., metastatic melanoma), renal cell carcinoma (e.g., metastatic renal cell carcinoma), leukemia, lymphoma (e.g., T cell lymphoma), and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer. Other preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. A highly preferred indication is infection (e.g., tuberculosis, infections associated with granulomatous</p>
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					<p>disease, and osteoporosis, and/or an infectious disease as described below under "Infectious Disease"). A highly preferred indication is AIDS. Additional highly preferred indications include suppression of immune reactions to transplanted organs and/or tissues, uveitis, psoriasis, and tropical spastic paraparesis. Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or "Cardiovascular Disorders"). Preferred indications also include anemia, pancytopenia, leukopenia, thrombocytopenia, Hodgkin's disease, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, granulomatous disease, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, asthma and allergy.</p>
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96	HEQBJ01	213	<p>Production of IL-13 and activation of T-cells.</p>	<p>Assays for production of IL-13 and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-13 and/or activation of T-cells. Exemplary assays for IL-13 production that may be used or routinely modified to test activity of polypeptides and antibodies of the invention (including agonists or antagonists of the invention) include, for example, assays such as disclosed and/or cited in: Grunig, G, et al., "Requirement for IL-13 independently of IL-4 in Experimental asthma" Science; 282: 2261-2263 (1998), and Wills-Karp M, et al., "Interleukin-13: central mediator of allergic asthma" Science; 282: 2258-2261 (1998); the contents of each of which are herein incorporated by reference in their entirety. Exemplary cells that may be</p>	<p>Highly preferred indications include allergy and asthma. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.</p>
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				<p>used according to these assays include Th2 cells. IL13, a Th2 type cytokine, is a potent stimulus for mucus production, airway hyper-responsiveness and allergic asthma. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6. Factors that induce differentiation and activation of Th2 cells play a major role in the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated in vitro culture under Th2 polarizing conditions using peripheral blood lymphocytes isolated from cord blood.</p>	
97	HBJHT01	214	<p>Production of IL-10 and activation of T-cells.</p>	<p>Assays for production of IL-10 and activation of T-cells are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to stimulate or inhibit production of IL-10 and/or activation of T-cells. Exemplary assays that may be used or routinely modified to assess the ability of</p>	<p>Highly preferred indications include allergy and asthma. Additional highly preferred indications include immune and hematopoietic disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis and/or as described below), immunodeficiencies (e.g., as</p>

				<p>polypeptides and antibodies of the invention (including agonists or antagonists of the invention) to modulate IL-10 production and/or T-cell proliferation include, for example, assays such as disclosed and/or cited in: Robinson, DS, et al., "Th-2 cytokines in allergic disease" Br Med Bull; 56 (4): 956-968 (2000), and Cohn, et al., "T-helper type 2 cell-directed therapy for asthma" Pharmacology & Therapeutics; 88: 187-196 (2000); the contents of each of which are herein incorporated by reference in their entirety. Exemplary cells that may be used according to these assays include Th2 cells. IL10 secreted from Th2 cells may be measured as a marker of Th2 cell activation. Th2 cells are a class of T cells that secrete IL4, IL10, IL13, IL5 and IL6. Factors that induce differentiation and activation of Th2 cells play a major role in the initiation and pathogenesis of allergy and asthma. Primary T helper 2 cells are generated via in vitro culture under Th2 polarizing conditions using</p>	described below), boosting a T cell-mediated immune response, and suppressing a T cell-mediated immune response.
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98	HAGDW20	215	Inhibition of squalene synthetase gene transcription.	<p>peripheral blood lymphocytes isolated from cord blood.</p> <p>Reporter Assay: construct contains regulatory and coding sequence of squalene synthetase, the first specific enzyme in the cholesterol biosynthetic pathway. See Jiang, et al., J. Biol. Chem. 268:12818-12824(1993), the contents of which are herein incorporated by reference in its entirety. Cells were treated with SID supernatants, and SEAP activity was measured after 72 hours. HepG2 is a human hepatocellular carcinoma cell line (ATCC HB-8065). See Knowles et al., Science. 209:497-9 (1980), the contents of which are herein incorporated by reference in its entirety.</p>	<p>Highly preferred indications include allergy, asthma, and rhinitis. Additional preferred indications include diabetes. Additional highly preferred indications include complications associated with diabetes (e.g., diabetic retinopathy, diabetic nephropathy, kidney disease (e.g., renal failure, nephropathy and/or other diseases and disorders as described in the "Renal Disorders" section below), diabetic neuropathy, nerve disease and nerve damage (e.g., due to diabetic neuropathy), blood vessel blockage, heart disease, stroke, impotence (e.g., due to diabetic neuropathy or blood vessel blockage), seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, endocrine disorders (as described in the "Endocrine Disorders" section below), neuropathy, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, and infection (e.g., infectious diseases and disorders as described in the "Infectious</p>
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98	HAGDW20	215	Activation or inhibition of transcription through NFkB response element in immune cells (such as basophils).	<p>This reporter assay measures activation or inhibition of the NFkB signaling pathway in Ku812 human basophil cell line. Assays for the activation or inhibition of transcription through the NFkB response element are well-known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to regulate NFkB transcription factors and modulate expression of immunomodulatory genes. NFkB is important in the pathogenesis of asthma. Exemplary assays for transcription through the NFkB response element that may be</p>	<p>Diseases" section below, especially of the urinary tract and skin). Highly preferred indications also include obesity, weight gain, and weight loss, as well as complications associated with obesity, weight gain, and weight loss. Preferred embodiments of the invention include methods of preventing, detecting, diagnosing, treating and/or ameliorating the above mentioned conditions, disorders, and diseases.</p> <p>Highly preferred indications include asthma, allergy, mastocytosis (a rare, heterogeneous disorder characterized by excessive accumulation of mast cells, and their proliferation and action in the skin, central nervous system, and other organs). Preferred indications also include hematopoietic and immunological disorders (e.g., as described below under "Immune Activity", and "Blood-Related Disorders"), infection (e.g., as described below under Infectious Disease), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below), and immunodeficiencies.</p>
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				<p>used or routinely modified to test NFkB-response element activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include assays disclosed in Berger et al., Gene 66:1-10 (1998); Cullen and Malm, Methods in Enzymol 216:362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85:6342-6346 (1988); Marone et al, Int Arch Allergy Immunol 114(3):207-17 (1997), the contents of each of which are herein incorporated by reference in its entirety. Cells were pretreated with SID supernatants or controls for 15-18 hours, and then 10 ng/mL of TNF was added to stimulate the NFkB reporter. SEAP activity was measured after 48 hours.</p> <p>Basophils that may be used according to these assays are publicly available (e.g., through the ATCC). Exemplary human basophil cell lines that may be used according to these assays include Ku812, originally established from a patient with chronic myelogenous leukemia. It is an immature prebasophilic</p>
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99	HTLJF11	216	Protection from Endothelial Cell Apoptosis.	<p>cell line that can be induced to differentiate into mature basophils. See, Kishi et al., Leuk Res. 9:381-390 (1985); Blom et al., Eur J Immunol. 22:2025-32 (1992), where the contents of each are herein incorporated by reference in its entirety.</p> <p>Caspase Apoptosis Rescue. Assays for caspase apoptosis rescue are well known in the art and may be used or routinely modified to assess the ability of the polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to inhibit caspase protease-mediated apoptosis. Exemplary assays for caspase apoptosis that may be used or routinely modified to test caspase apoptosis rescue of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Romeo et al., Cardiovasc Res 45(3): 788-794 (2000); Messmer et al., Br J Pharmacol 127(7): 1633-1640 (1999); and J Atheroscler Thromb 3(2): 75-80 (1996); the</p>	<p>A highly preferred embodiment of the invention includes a method for stimulating endothelial cell growth. An alternative highly preferred embodiment of the invention includes a method for inhibiting endothelial cell growth. A highly preferred embodiment of the invention includes a method for stimulating endothelial cell proliferation. An alternative highly preferred embodiment of the invention includes a method for inhibiting endothelial cell proliferation. A highly preferred embodiment of the invention includes a method for stimulating endothelial cell growth. An alternative highly preferred embodiment of the invention includes a method for inhibiting endothelial cell</p>
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				<p>growth. A highly preferred embodiment of the invention includes a method for stimulating apoptosis of endothelial cells. An alternative highly preferred embodiment of the invention includes a method for inhibiting (e.g., decreasing) apoptosis of endothelial cells. A highly preferred embodiment of the invention includes a method for stimulating angiogenesis. An alternative highly preferred embodiment of the invention includes a method for inhibiting angiogenesis. A highly preferred embodiment of the invention includes a method for reducing cardiac hypertrophy. An alternative highly preferred embodiment of the invention includes a method for inducing cardiac hypertrophy. Highly preferred indications include neoplastic diseases (e.g., as described below under "Hyperproliferative Disorders"), and disorders of the cardiovascular system (e.g., heart disease, congestive heart failure, hypertension, aortic stenosis, cardiomyopathy,</p>
				<p>contents of each of which are herein incorporated by reference in its entirety. Endothelial cells that may be used according to these assays are publicly available (e.g., through commercial sources). Exemplary endothelial cells that may be used according to these assays include bovine aortic endothelial cells (bAEC), which are an example of endothelial cells which line blood vessels and are involved in functions that include, but are not limited to, angiogenesis, vascular permeability, vascular tone, and immune cell extravasation.</p>

					<p>valvular regurgitation, left ventricular dysfunction, atherosclerosis and atherosclerotic vascular disease, diabetic nephropathy, intracardiac shunt, cardiac hypertrophy, myocardial infarction, chronic hemodynamic overload, and/or as described below under “Cardiovascular Disorders”).</p> <p>Highly preferred indications include cardiovascular, endothelial and/or angiogenic disorders (e.g., systemic disorders that affect vessels such as diabetes mellitus, as well as diseases of the vessels themselves, such as of the arteries, capillaries, veins and/or lymphatics). Highly preferred are indications that stimulate angiogenesis and/or cardiovascularization. Highly preferred are indications that inhibit angiogenesis and/or cardiovascularization.</p> <p>Highly preferred indications include antiangiogenic activity to treat solid tumors, leukemias, and Kaposi's sarcoma, and retinal disorders. Highly preferred indications include</p>
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					neoplasms and cancer, such as, Kaposi's sarcoma, hemangioma (capillary and cavernous), glomus tumors, telangiectasia, bacillary angiomatosis, hemangioendothelioma, angiosarcoma, haemangiopericytoma, lymphangioma, lymphangiosarcoma. Highly preferred indications also include cancers such as, prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver, and urinary cancer. Preferred indications include benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia. Highly preferred indications also include arterial disease, such as, atherosclerosis, hypertension, coronary artery disease, inflammatory vasculitides, Reynaud's disease and Reynaud's phenomenon, aneurysms, restenosis; venous and lymphatic disorders such as thrombophlebitis, lymphangitis, and lymphedema; and other vascular disorders such as peripheral vascular disease, and
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					<p>cancer. Highly preferred indications also include trauma such as wounds, burns, and injured tissue (e.g., vascular injury such as, injury resulting from balloon angioplasty, and atherosclerotic lesions), implant fixation, scarring, ischemia reperfusion injury, rheumatoid arthritis, cerebrovascular disease, renal diseases such as acute renal failure, and osteoporosis. Additional highly preferred indications include stroke, graft rejection, diabetic or other retinopathies, thrombotic and coagulative disorders, vasculitis, lymph angiogenesis, sexual disorders, age-related macular degeneration, and treatment/prevention of endometriosis and related conditions. Additional highly preferred indications include fibromas, heart disease, cardiac arrest, heart valve disease, and vascular disease. Preferred indications include blood disorders (e.g., as described below under "Immune Activity", "Blood-Related Disorders", and/or</p>
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99	HTLIF11	216	Production of RANTES in endothelial cells (such as human umbilical vein endothelial cells (HUVEC))	RANTES FMAT. Assays for immunomodulatory proteins that induce chemotaxis of T cells, monocytes, and eosinophils are well known in the art and may be used or routinely modified to assess the ability of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) to mediate immunomodulation, induce chemotaxis, and/or mediate humoral or cell-mediated immunity. Exemplary assays that test for immunomodulatory proteins evaluate the production	<p>"Cardiovascular Disorders"). Preferred indications include autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and/or as described below) and immunodeficiencies (e.g., as described below). Additional preferred indications include inflammation and inflammatory disorders (such as acute and chronic inflammatory diseases, e.g., inflammatory bowel disease and Crohn's disease), and pain management.</p> <p>Preferred indications include allergy and asthma. Highly preferred indications include immunological disorders such as described herein under the heading "Immune Activity" and/or "Blood-Related Disorders" (particularly including, but not limited to, immune disorders involving endothelial cells). Highly preferred embodiments of the invention include methods of preventing, detecting, diagnosing, treating and/or ameliorating disorders of the immune system (particularly including, but not limited to, immune disorders involving endothelial cells). (HUVEC cells are human umbilical vein endothelial cells).</p>
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				<p>of cytokines, such as RANTES, and the induction of chemotactic responses in immune cells. Such assays that may be used or routinely modified to test immunomodulatory activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Miraglia et al., J Biomolecular Screening 4:193-204 (1999); Cocchi et al., Science 270(5243):1811-1815 (1995); and Robinson et al., Clin Exp Immunol 101(3):398-407 (1995), the contents of each of which are herein incorporated by reference in its entirety. Endothelial cells that may be used according to these assays are publicly available (e.g., through the ATCC). Exemplary endothelial cells that may be used according to these assays include human umbilical vein endothelial cells (HUVEC), which are endothelial cells which line venous blood vessels, and are involved in functions that include, but are not limited to, angiogenesis, vascular permeability, vascular tone, and</p>
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Table 1E: Polynucleotides encoding polypeptides of the present invention can be used in assays to test for one or more biological activities. One such biological activity which may be tested includes the ability of polynucleotides and polypeptides of the invention to stimulate up-regulation or down-regulation of expression of particular genes and proteins. Hence, if polynucleotides and polypeptides of the present invention exhibit activity in altering particular gene and protein expression patterns, it is likely that these polynucleotides and polypeptides of the present invention may be involved in, or capable of effecting changes in, diseases associated with the altered gene and protein expression profiles. Hence, polynucleotides, polypeptides, or antibodies of the present invention could be used to treat said associated diseases.

TaqMan® assays may be performed to assess the ability of polynucleotides (and polypeptides they encode) to alter the expression pattern of particular "target" genes. TaqMan® reactions are performed to evaluate the ability of a test agent to induce or repress expression of specific genes in different cell types. TaqMan® gene expression quantification assays ("TaqMan® assays") are well known to, and routinely performed by, those of ordinary skill in the art. TaqMan® assays are performed in a two step reverse transcription / polymerase chain reaction (RT-PCR). In the first (RT) step, cDNA is reverse transcribed from total RNA samples using random hexamer primers. In the second (PCR) step, PCR products are synthesized from the cDNA using gene specific primers.

To quantify gene expression the Taqman® PCR reaction exploits the 5' nuclease activity of AmpliTaq Gold® DNA Polymerase to cleave a Taqman® probe (distinct from the primers) during PCR. The Taqman® probe contains a reporter dye at the 5'-end of the probe and a quencher dye at the 3' end of the probe. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites. AmpliTaq Fold DNA Polymerase then cleaves the probe between the reporter and quencher when the probe hybridizes to the target, resulting in increased fluorescence of the reporter (see Figure 2). Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye.

After the probe fragments are displaced from the target, polymerization of the strand continues. The 3'-end of the probe is blocked to prevent extension of the probe during PCR. This process occurs in every cycle and does not interfere with the exponential accumulation of product. The increase in fluorescence signal is detected only if the target sequence is complementary to the probe and is amplified during PCR. Because of these requirements, any nonspecific amplification is not detected.

For test sample preparation, vector controls or constructs containing the coding sequence for the gene of interest are transfected into cells, such as for example 293T cells, and supernatants collected after 48 hours. For cell treatment and RNA isolation, multiple primary human cells or human cell lines are used; such cells may include but are not limited to, Normal Human Dermal Fibroblasts, Aortic Smooth Muscle, Human Umbilical Vein Endothelial Cells, HepG2, Daudi, Jurkat, U937, Caco, and THP-1 cell lines. Cells are plated in growth media and growth is arrested by culturing without media change for 3 days, or by switching cells to low serum media and incubating overnight. Cells are treated for 1, 6, or 24 hours with either vector control supernatant or sample supernatant (or purified/partially purified protein preparations in buffer). Total RNA is isolated; for example, by using Trizol extraction or by using the Ambion RNAqueous(TM)-4PCR RNA isolation system. Expression levels of multiple genes are analyzed using TAQMAN, and expression in the test sample is compared to control vector samples to identify genes induced or repressed. Each of the above described techniques are well known to, and routinely performed by, those of ordinary skill in the art.

Table 1E indicates particular disease classes and preferred indications for which polynucleotides, polypeptides, or antibodies of the present invention may be used in detecting, diagnosing, preventing, treating and/or ameliorating said diseases and disorders based on "target" gene expression patterns which may be up- or down-regulated by polynucleotides (and the encoded polypeptides) corresponding to each indicated cDNA Clone ID (shown in Table 1E, Column 2).

Thus, in preferred embodiments, the present invention encompasses a method of detecting, diagnosing, preventing, treating, and/or ameliorating a disease or disorder listed in the "Disease Class" and/or "Preferred Indication" columns of Table 1E; comprising administering to a patient in which such detection, diagnosis, prevention, or treatment is desired a protein, nucleic acid, or antibody of the invention (or fragment or variant thereof) in an amount effective to detect, diagnose, prevent, treat, or ameliorate the disease or disorder. The first and second columns of Table 1D show the "Gene No." and "cDNA Clone ID No.", respectively, indicating certain nucleic acids and proteins (or antibodies against the same) of the invention (including polynucleotide, polypeptide, and antibody fragments or variants thereof) that may be used in detecting, diagnosing, preventing, treating, or ameliorating the disease(s) or disorder(s) indicated in the corresponding row in the "Disease Class" or "Preferred Indication" Columns of Table 1E.

In another embodiment, the present invention also encompasses methods of detecting, diagnosing, preventing, treating, or ameliorating a disease or disorder listed in the "Disease Class" or "Preferred Indication" Columns of Table 1E; comprising administering to a patient combinations of the proteins, nucleic acids, or antibodies of the invention (or fragments or variants thereof), sharing similar indications as shown in the corresponding rows in the "Disease Class" or "Preferred Indication" Columns of Table 1E.

The "Disease Class" Column of Table 1E provides a categorized descriptive heading for diseases, disorders, and/or conditions (more fully described below) that may be detected, diagnosed, prevented, treated, or ameliorated by a protein, nucleic acid, or antibody of the invention (or fragment or variant thereof).

5 The "Preferred Indication" Column of Table 1E describes diseases, disorders, and/or conditions that may be detected, diagnosed, prevented, treated, or ameliorated by a protein, nucleic acid, or antibody of the invention (or fragment or variant thereof).

 The "Cell Line" and "Exemplary Targets" Columns of Table 1E indicate particular cell lines and target genes, respectively, which may show altered gene expression patterns (i.e., up- or
10 down-regulation of the indicated target gene) in Taqman assays, performed as described above, utilizing polynucleotides of the cDNA Clone ID shown in the corresponding row. Alteration of expression patterns of the indicated "Exemplary Target" genes is correlated with a particular "Disease Class" and/or "Preferred Indication" as shown in the corresponding row under the respective column headings.

15 The "Exemplary Accessions" Column indicates GenBank Accessions (available online through the National Center for Biotechnology Information (NCBI) at <http://www.ncbi.nlm.nih.gov/>) which correspond to the "Exemplary Targets" shown in the adjacent row.

 The recitation of "Cancer" in the "Disease Class" Column indicates that the
20 corresponding nucleic acid and protein, or antibody against the same, of the invention (or fragment or variant thereof) may be used for example, to detect, diagnose, prevent, treat, and/or ameliorate neoplastic diseases and/or disorders (e.g., leukemias, cancers, etc., as described below under "Hyperproliferative Disorders").

 The recitation of "Immune" in the "Disease Class" column indicates that the
25 corresponding nucleic acid and protein, or antibody against the same, of the invention (or fragment or variant thereof), may be used for example, to detect, diagnose, prevent, treat, and/or ameliorate diseases and/or disorders relating to neoplastic diseases (e.g., as described below under "Hyperproliferative Disorders"), blood disorders (e.g., as described below under "Immune Activity" "Cardiovascular Disorders" and/or "Blood-Related Disorders"), and infections (e.g., as
30 described below under "Infectious Disease").

 The recitation of "Angiogenesis" in the "Disease Class" column indicates that the corresponding nucleic acid and protein, or antibody against the same, of the invention (or fragment or variant thereof), may be used for example, to detect, diagnose, treat, prevent, and/or ameliorate diseases and/or disorders relating to neoplastic diseases (e.g., as described below under
35 "Hyperproliferative Disorders"), diseases and/or disorders of the cardiovascular system (e.g., as described below under "Cardiovascular Disorders"), diseases and/or disorders involving cellular and genetic abnormalities (e.g., as described below under "Diseases at the Cellular Level"),

diseases and/or disorders involving angiogenesis (e.g., as described below under "Anti-Angiogenesis Activity"), to promote or inhibit cell or tissue regeneration (e.g., as described below under "Regeneration"), or to promote wound healing (e.g., as described below under "Wound Healing and Epithelial Cell Proliferation").

5 The recitation of "Diabetes" in the "Disease Class" column indicates that the corresponding nucleic acid and protein, or antibody against the same, of the invention (or fragment or variant thereof), may be used for example, to detect, diagnose, treat, prevent, and/or ameliorate diabetes (including diabetes mellitus types I and II), as well as diseases and/or disorders associated with, or consequential to, diabetes (e.g. as described below under "Endocrine Disorders," "Renal
10 Disorders," and "Gastrointestinal Disorders").

Table 1E

Gene No.	cDNA Clone ID	Disease Class	Preferred Indications:	Cell Line:	Exemplary Targets	Exemplary Accessions
76	HTEEW69	Immune	Highly preferred indications include immunological disorders such as described herein under the heading "Immune Activity" and/or "Blood-Related Disorders" (particularly including, but not limited to, immune disorders involving muscle tissues and the cardiovascular system (e.g. heart, lungs, circulatory system)). Highly preferred embodiments of the invention include methods of preventing, detecting, diagnosing, treating and/or ameliorating disorders of the immune system (particularly including, but not limited to, immune disorders involving muscle tissue or the cardiovascular system). (AOSMC cells are human aortic smooth muscle cells).	AOSMC	CCR7 CXCR3 Rag2 VLA4	gb X84702 HSDN ABLR2 gb Z79783 HSCCK RL2 gb AY011962 AY 011962 gb X16983 HSINT AL4
76	HTEEW69	Immune	Highly preferred indications include immunological disorders such as described herein under the heading "Immune Activity" and/or "Blood-Related Disorders" (particularly including, but not limited to, immune disorders involving the cells of the gastrointestinal tract). Highly preferred embodiments of the invention include methods of preventing, detecting, diagnosing, treating and/or ameliorating disorders of the immune system (particularly including, but not limited to, immune disorders involving cells of the gastrointestinal tract). (The Caco-2 cell line is a human colorectal adenocarcinoma cell line available through the ATCC as cell line number HTB-37).	Caco-2	TNF	gb AJ270944 HSA 27094
76	HTEEW69	Immune	Highly preferred indications include immunological disorders such as described herein under the heading "Immune Activity" and/or "Blood-Related Disorders" (particularly including, but not limited to, immune disorders involving the B-cells). Highly preferred embodiments of the invention include methods of preventing, detecting, diagnosing, treating and/or ameliorating disorders of the immune system (particularly including, but not limited to, immune disorders involving B-cells). (The Daudi cell line is a human B lymphoblast cell line available through the ATCC as cell line number CCL-213).	Daudi	GATA3 ICAM TNF	gb X55037 HSGA TA3 gb X06990 HSICA M1 gb AJ270944 HSA 27094

76	HTEEW69	Immune	Highly preferred indications include immunological disorders such as described herein under the heading "Immune Activity" and/or "Blood-Related Disorders" (particularly including, but not limited to, immune disorders involving epithelial cells or the renal system). Highly preferred embodiments of the invention include methods of preventing, detecting, diagnosing, treating and/or ameliorating disorders of the immune system (particularly including, but not limited to, immune disorders involving epithelial cells or the renal system). (The 293 cell line is a human embryonal kidney epithelial cell line available through the ATCC as cell line number CRL-1573).	HEK293	TNF	gb AJ270944 HSA 27094
76	HTEEW69	Immune	Highly preferred indications include immunological disorders such as described herein under the heading "Immune Activity" and/or "Blood-Related Disorders" (particularly including, but not limited to, immune disorders involving cells of the hepatic system). Highly preferred embodiments of the invention include methods of preventing, detecting, diagnosing, treating and/or ameliorating disorders of the immune system (particularly including, but not limited to, immune disorders involving cells of the hepatic system).	Liver	ICAM	gb X06990 HSICA M1
76	HTEEW69	Immune	Highly preferred indications include immunological disorders such as described herein under the heading "Immune Activity" and/or "Blood-Related Disorders" (particularly including, but not limited to, immune disorders involving the skin). Highly preferred embodiments of the invention include methods of preventing, detecting, diagnosing, treating and/or ameliorating disorders of the immune system (particularly including, but not limited to, immune disorders involving dermal fibroblasts).	NHDF	CIS3 TNF	gb AB006967 AB 006967 gb AJ270944 HSA 27094
76	HTEEW69	Immune	Highly preferred indications include immunological disorders such as described herein under the heading "Immune Activity" and/or "Blood-Related Disorders" (particularly including, but not limited to, immune disorders involving the central nervous system). Highly preferred embodiments of the invention include methods of preventing, detecting, diagnosing, treating and/or ameliorating disorders of the immune system (particularly including, but not limited to, immune disorders involving the central nervous system).	SK-N-MC neuroblastoma	TNF VCAM	gb AJ270944 HSA 27094 gb A30922 A3092 2

76	HTEEW69	Immune	immune system (particularly including, but not limited to, immune disorders involving the central nervous system). (The SK-N-MC neuroblastoma cell line is a cell line derived from human brain tissue and is available through the ATCC as cell line number HTB-10). Highly preferred indications include immunological disorders such as described herein under the heading "Immune Activity" and/or "Blood-Related Disorders" (particularly including, but not limited to, immune disorders involving monocytes). Highly preferred embodiments of the invention include methods of preventing, detecting, diagnosing, treating and/or ameliorating disorders of the immune system (particularly including, but not limited to, immune disorders involving monocytes). (The THP1 cell line is a human monocyte cell line available through the ATCC as cell line number TIB-202).	THP1	CD25 CD40 GATA3 LTBR Rag1	gb X03137 HSIL2 RG7 gb AJ300189 HSA 30018 gb X55037 HSGA TA3 gb AK027080 AK 027080 gb M29474 HUM RAG1	
76	HTEEW69	Immune	Highly preferred indications include immunological disorders such as described herein under the heading "Immune Activity" and/or "Blood-Related Disorders" (particularly including, but not limited to, immune disorders involving monocytes). Highly preferred embodiments of the invention include methods of preventing, detecting, diagnosing, treating and/or ameliorating disorders of the immune system (particularly including, but not limited to, immune disorders involving monocytes). (The U937 cell line is a human monocyte cell line available through the ATCC as cell line number CRL-1593.2).	U937	IL1B TNF	gb X02532 HSIL1 BR gb AJ270944 HSA 27094	

Table 2 further characterizes certain encoded polypeptides of the invention, by providing the results of comparisons to protein and protein family databases. The first column provides a unique clone identifier, "Clone ID NO:", corresponding to a cDNA clone disclosed in Table 1A and/or Table 1B. The second column provides the unique contig identifier, "Contig ID:" which allows correlation with the information in Table 1B. The third column provides the sequence identifier, "SEQ ID NO:", for the contig polynucleotide sequences. The fourth column provides the analysis method by which the homology/identity disclosed in the Table was determined. The fifth column provides a description of the PFAM/NR hit identified by each analysis. Column six provides the accession number of the PFAM/NR hit disclosed in the fifth column. Column seven, score/percent identity, provides a quality score or the percent identity, of the hit disclosed in column five. Comparisons were made between polypeptides encoded by polynucleotides of the invention and a non-redundant protein database (herein referred to as "NR"), or a database of protein families (herein referred to as "PFAM"), as described below.

The NR database, which comprises the NBRF PIR database, the NCBI GenPept database, and the SIB SwissProt and TrEMBL databases, was made non-redundant using the computer program nrdb2 (Warren Gish, Washington University in Saint Louis). Each of the polynucleotides shown in Table 1B, column 3 (e.g., SEQ ID NO:X or the 'Query' sequence) was used to search against the NR database. The computer program BLASTX was used to compare a 6-frame translation of the Query sequence to the NR database (for information about the BLASTX algorithm please see Altshul et al., J. Mol. Biol. 215:403-410 (1990), and Gish and States, Nat. Genet. 3:266-272 (1993). A description of the sequence that is most similar to the Query sequence (the highest scoring 'Subject') is shown in column five of Table 2 and the database accession number for that sequence is provided in column six. The highest scoring 'Subject' is reported in Table 2 if (a) the estimated probability that the match occurred by chance alone is less than 1.0×10^{-7} , and (b) the match was not to a known repetitive element. BLASTX returns alignments of short polypeptide segments of the Query and Subject sequences that share a high degree of similarity; these segments are known as High-Scoring Segment Pairs or HSPs. Table 2 reports the degree of similarity between the Query and the Subject for each HSP as a percent identity in Column 7. The percent identity is determined by dividing the number of exact matches between the two aligned sequences in the HSP, dividing by the number of Query amino acids in the HSP and multiplying by 100. The polynucleotides of SEQ ID NO:X which encode the polypeptide sequence that generates an HSP are delineated by columns 8 and 9 of Table 2.

The PFAM database, PFAM version 2.1, (Sonnhammer, Nucl. Acids Res., 26:320-322, 1998)) consists of a series of multiple sequence alignments; one alignment for each protein family.

Each multiple sequence alignment is converted into a probability model called a Hidden Markov Model, or HMM, that represents the position-specific variation among the sequences that make up the multiple sequence alignment (see, e.g., Durbin, et al., *Biological sequence analysis: probabilistic models of proteins and nucleic acids*, Cambridge University Press, 1998 for the theory of HMMs). The program HMMER version 1.8 (Sean Eddy, Washington University in Saint Louis) was used to compare the predicted protein sequence for each Query sequence (SEQ ID NO:Y in Table 1B) to each of the HMMs derived from PFAM version 2.1. A HMM derived from PFAM version 2.1 was said to be a significant match to a polypeptide of the invention if the score returned by HMMER 1.8 was greater than 0.8 times the HMMER 1.8 score obtained with the most distantly related known member of that protein family. The description of the PFAM family which shares a significant match with a polypeptide of the invention is listed in column 5 of Table 2, and the database accession number of the PFAM hit is provided in column 6. Column 7 provides the score returned by HMMER version 1.8 for the alignment. Columns 8 and 9 delineate the polynucleotides of SEQ ID NO:X which encode the polypeptide sequence which show a significant match to a PFAM protein family.

As mentioned, columns 8 and 9 in Table 2, "NT From" and "NT To", delineate the polynucleotides of "SEQ ID NO:X" that encode a polypeptide having a significant match to the PFAM/NR database as disclosed in the fifth column. In one embodiment, the invention provides a protein comprising, or alternatively consisting of, a polypeptide encoded by the polynucleotides of SEQ ID NO:X delineated in columns 8 and 9 of Table 2. Also provided are polynucleotides encoding such proteins, and the complementary strand thereto.

The nucleotide sequence SEQ ID NO:X and the translated SEQ ID NO:Y are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, the nucleotide sequences of SEQ ID NO:X are useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in ATCC Deposit No:Z. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling immediate applications in chromosome mapping, linkage analysis, tissue identification and/or typing, and a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used to generate antibodies which bind specifically to these polypeptides, or fragments thereof, and/or to the polypeptides encoded by the cDNA clones identified in, for example, Table 1A and/or 1B.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts

in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

5 Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X, and a predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing cDNA ATCC Deposit No:Z (e.g., as set forth in columns 2 and 3 of Table 1A and/or as set forth, for example, in Table 1B, 6, and 7). The
10 nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods. Further, techniques known in the art can be used to verify the nucleotide sequences of SEQ ID NO:X. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the
15 protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

Table 2

cDNA Clone ID	Contig ID:	SEQ ID NO: X	Analysis Method	PFam/NR Description	PFam/NR Accession Number	Score/Percent Identity	NT From	NT To
HADMB15	847116	15	WUblastx .64	(Q9BVH1) SIMILAR TO DLXIN-1.	Q9BVH1	100%	8	109
HAGCC87	638587	17	WUblastx .64	(Q9BGW3) HYPOTHETICAL 13.5 KDA PROTEIN.	Q9BGW3	65% 36% 57%	992 54 801	1105 116 980
HAGHN57	773286	18	WUblastx .64	(O60416) WUGSC:H_RG276003.2 PROTEIN.	O60416	98%	65	1444
HAQCE11	633730	22	WUblastx .64	(Q24333) ELASTIN LIKE PROTEIN (FRAGMENT).	Q24333	95%	61	132
HBAGD86	838799	23	WUblastx .64	(Q14287) HYPOTHETICAL PROTEIN (FRAGMENT).	Q14287	37%	801	559
HBGBC29	691473	24	WUblastx .64	(O60513) BETA-1,4-GALACTOSYLTRANSFERASE 4 (EC 2.4.1.-) (BET	B4G4_HUMAN	61% 98%	1 65	78 1021
HBJAB02	837309	25	WUblastx .64	(Q9NXT6) CDNA FLJ20062 FIS, CLONE COL01508.	Q9NXT6	70%	2	1210
HBMUH74	866160	26	WUblastx .64	(Q9NVW8) CDNA FLJ10462 FIS, CLONE NT2RP1001494, WEAKLY SIMILAR TO MAL	Q9NVW8	100%	11	427
HBNAX40	834801	27	WUblastx .64	(Q9H2K2) TANKYRASE-LIKE PROTEIN (TANKYRASE 2).	Q9H2K2	100% 100%	1 221	201 481
HBXCX15	637542	28	WUblastx .64	(Q9GMX5) HYPOTHETICAL 12.9 KDA PROTEIN.	Q9GMX5	41% 52%	726 578	827 730
HCDBO32	831942	29	WUblastx .64	(AAH17472) Hypothetical 21.3 kDa protein.	AAH17472	69% 100%	643 239	801 583
HCEFZ82	831745	31	WUblastx	(Q9BV23) SIMILAR TO LIPASE PROTEIN.	Q9BV23	95%	594	782

HCUCF89	637986	32	.64 WUblastx	(Q9P147) PRO2822.		Q9P147	100%	17	604
HDPDI72	897277	35	.64 WUblastx	adult-specific brush border protein - rabbit		pir C45665 C45665	64% 83%	180 11	230 100
HDPIE44	899328	37	.64 WUblastx	(Q9D666) 4632417G13RIK PROTEIN.		Q9D666	62%	102	2453
HDPIU94	813352	38	.64 WUblastx	(Q9BVF7) SIMILAR TO HYPOTHETICAL PROTEIN FLJ10422.		Q9BVF7	99%	63	1703
HE6CS65	762960	41	.64 WUblastx	(Q9H7C6) CDNA: FLJ21047 FIS, CLONE CAS00253.		Q9H7C6	98%	938	1378
HE8BQ49	589443	42	.64 WUblastx	hypothetical protein - human transposon MER37		pir S72482 S72482	75% 64%	343 105	474 248
HE9CY05	834826	43	.64 WUblastx	(Q9CX63) 6030468B19RIK PROTEIN.		Q9CX63	48% 57%	434 55	742 426
HEAAW94	847340	44	.64 WUblastx	(Q9UEV9) ACTIN-BINDING PROTEIN HOMOLOG ABP-278.		Q9UEV9	94%	285	890
HEBFR46	847064	45	.64 WUblastx	(Q9NX85) CDNA FLJ20378 FIS, CLONE KAIA0536.		Q9NX85	80% 84%	1111 1265	1022 1110
HFEBO17	852218	48	.64 WUblastx	(BAB55130) CDNA FLJ14559 fis, clone NT2RM2001998.		BAB55130	100% 91%	523 606	624 809
HFJIA29	839206	49	.64 WUblastx	(Q9UHT1) PRO1902 PROTEIN.		Q9UHT1	46% 59%	889 1026	806 880
HGBER72	826710	51	.64 WUblastx	(Q9H387) PRO2550.		Q9H387	71% 78% 77%	1061 1104 1237	969 1063 1103
HHSDI53	862028	55	.64 WUblastx	(Q9H387) PRO2550.		Q9H387	70% 71% 75%	1108 1241 1276	935 1107 1241
HISBA38	561711	56	.64 WUblastx	(Q9H387) PRO2550.		Q9H387	53% 53%	919 996	836 907

HKABU43	838573	58	WUblastx .64	(AAH03633) Translocase of outer mitochondrial membr	AAH03633	51%	842	687
HLYGY91	658703	60	WUblastx .64	(Q9H8N0) CDNA FLJ13386 FIS, CLONE PLACE1001104, WEAKLY SIMILAR TO MYO	Q9H8N0	94%	221	391
HMWJF53	758158	64	WUblastx .64	(Q9GZU7) NUCLEAR LIM INTERACTOR-INTERACTING FACTOR.	Q9GZU7	91% 100%	3 154	170 720
HNECL22	799541	65	WUblastx .64	(Q9P0J2) MITOCHONDRIAL SOLUTE CARRIER.	Q9P0J2	94%	1771	2331
HNFAC50	815676	66	WUblastx .64	(Q9H286) SEROLOGICALLY DEFINED BREAST CANCER ANTIGEN NY-BR-20 (FRAGME	Q9H286	100%	425	282
HNHCT47	634691	71	WUblastx .64	(Q9H728) CDNA: FLJ21463 FIS, CLONE COL04765.	Q9H728	46% 56%	434 621	396 448
HNHK174	777856	72	WUblastx .64	(Q9BGX7) HYPOTHETICAL 13.0 KDA PROTEIN.	Q9BGX7	64%	350	541
HOUDE92	580866	74	WUblastx .64	(Q9HBT2) HYPOTHETICAL 17.2 KDA PROTEIN.	Q9HBT2	96%	21	245
HOUFS04	771564	75	WUblastx .64	(Q9VN45) CG12001 PROTEIN.	Q9VN45	32% 39% 26%	1362 915 141	1982 1106 380
HOUHI25	888279	76	WUblastx .64	(O95003) WUGSC:H_DJ0593H12.2 PROTEIN.	O95003	94%	73	783
HPCAL26	762822	77	WUblastx .64	(O95084) SERINE PROTEASE (HYPOTHETICAL 43.0 KDA PROTEIN) (PROTEASE, S	O95084	98% 76%	398 135	640 497
HSAVA08	580870	79	WUblastx .64	(Q9BGW3) HYPOTHETICAL 13.5 KDA PROTEIN.	Q9BGW3	57% 42% 63% 66%	949 926 796 1059	896 792 764 934
HSHAX04	812178	80	WUblastx .64	peptidylprolyl isomerase (EC 5.2.1.8) A - human	pir S66681 S66681	96%	14	916
HSRFD18	840771	83	WUblastx	(Q9H941) CDNA FLJ13033 FIS, CLONE	Q9H941	100%	437	559

RACE Protocol For Recovery of Full-Length Genes

Partial cDNA clones can be made full-length by utilizing the rapid amplification of cDNA ends (RACE) procedure described in Frohman, M.A., et al., Proc. Nat'l. Acad. Sci. USA, 85:8998-9002 (1988). A cDNA clone missing either the 5' or 3' end can be reconstructed to include the
5 absent base pairs extending to the translational start or stop codon, respectively. In some cases, cDNAs are missing the start codon of translation, therefore. The following briefly describes a modification of this original 5' RACE procedure. Poly A⁺ or total RNA is reverse transcribed with Superscript II (Gibco/BRL) and an antisense or complementary primer specific to the cDNA sequence. The primer is removed from the reaction with a Microcon Concentrator (Amicon). The
10 first-strand cDNA is then tailed with dATP and terminal deoxynucleotide transferase (Gibco/BRL). Thus, an anchor sequence is produced which is needed for PCR amplification. The second strand is synthesized from the dA-tail in PCR buffer, Taq DNA polymerase (Perkin-Elmer Cetus), an oligo-dT primer containing three adjacent restriction sites (XhoI, SalI and ClaI) at the 5' end and a primer containing just these restriction sites. This double-stranded cDNA is PCR
15 amplified for 40 cycles with the same primers as well as a nested cDNA-specific antisense primer. The PCR products are size-separated on an ethidium bromide-agarose gel and the region of gel containing cDNA products the predicted size of missing protein-coding DNA is removed. cDNA is purified from the agarose with the Magic PCR Prep kit (Promega), restriction digested with XhoI or SalI, and ligated to a plasmid such as pBluescript SKII (Stratagene) at XhoI and EcoRV
20 sites. This DNA is transformed into bacteria and the plasmid clones sequenced to identify the correct protein-coding inserts. Correct 5' ends are confirmed by comparing this sequence with the putatively identified homologue and overlap with the partial cDNA clone. Similar methods known in the art and/or commercial kits are used to amplify and recover 3' ends.

Several quality-controlled kits are commercially available for purchase. Similar reagents
25 and methods to those above are supplied in kit form from Gibco/BRL for both 5' and 3' RACE for recovery of full-length genes. A second kit is available from Clontech, which is a modification of a related technique, SLIC (single-stranded ligation to single-stranded cDNA), developed by Dumas et al., Nucleic Acids Res., 19:5227-32 (1991). The major differences in procedure are that the RNA is alkaline hydrolyzed after reverse transcription and RNA ligase is used to join a
30 restriction site-containing anchor primer to the first-strand cDNA. This obviates the necessity for the dA-tailing reaction that results in a polyT stretch that is difficult to sequence past.

An alternative to generating 5' or 3' cDNA from RNA is to use cDNA library double-stranded DNA. An asymmetric PCR-amplified antisense cDNA strand is synthesized with an antisense cDNA-specific primer and a plasmid-anchored primer. These primers are removed and a

symmetric PCR reaction is performed with a nested cDNA-specific antisense primer and the plasmid-anchored primer.

RNA Ligase Protocol For Generating The 5' or 3' End Sequences To Obtain Full Length Genes

5 Once a gene of interest is identified, several methods are available for the identification of the 5' or 3' portions of the gene that may not be present in the original cDNA plasmid. These methods include, but are not limited to, filter probing, clone enrichment using specific probes and protocols similar and identical to 5' and 3' RACE. While the full-length gene may be present in the library and can be identified by probing, a useful method for generating the 5' or 3' end is to use
10 the existing sequence information from the original cDNA to generate the missing information. A method similar to 5' RACE is available for generating the missing 5' end of a desired full-length gene. (This method was published by Fromont-Racine et al., Nucleic Acids Res., 21(7):1683-1684 (1993)). Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcript and a primer set containing a primer
15 specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest, is used to PCR amplify the 5' portion of the desired full length gene which may then be sequenced and used to generate the full length gene. This method starts with total RNA isolated from the desired source, poly A RNA may be used but is not a prerequisite for this procedure. The RNA preparation may then be treated with phosphatase if necessary to eliminate 5' phosphate
20 groups on degraded or damaged RNA, which may interfere with the later RNA ligase step. The phosphatase if used is then inactivated and the RNA is treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase. This modified RNA preparation can then be used as a
25 template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction can then be used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the relevant gene.

30 The present invention also relates to vectors or plasmids, which include such DNA sequences, as well as the use of the DNA sequences. The material deposited with the ATCC (e.g., as described in columns 2 and 3 of Table 1A, and/or as set forth in Table 1B, Table 6, or Table 7) is a mixture of cDNA clones derived from a variety of human tissue and cloned in either a plasmid vector or a phage vector, as described, for example, in Table 1A and Table 7. These deposits are
35 referred to as "the deposits" herein. The tissues from which some of the clones were derived are

listed in Table 7, and the vector in which the corresponding cDNA is contained is also indicated in Table 7. The deposited material includes cDNA clones corresponding to SEQ ID NO:X described, for example, in Table 1A and/or Table 1B (ATCC Deposit No:Z). A clone which is isolatable from the ATCC Deposits by use of a sequence listed as SEQ ID NO:X, may include the entire
5 coding region of a human gene or in other cases such clone may include a substantial portion of the coding region of a human gene. Furthermore, although the sequence listing may in some instances list only a portion of the DNA sequence in a clone included in the ATCC Deposits, it is well within the ability of one skilled in the art to sequence the DNA included in a clone contained in the ATCC Deposits by use of a sequence (or portion thereof) described in, for example Tables
10 1A and/or Table 1B or Table 2, by procedures hereinafter further described, and others apparent to those skilled in the art.

Also provided in Table 1A and Table 7 is the name of the vector which contains the cDNA clone. Each vector is routinely used in the art. The following additional information is provided for convenience.

15 Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., *Nucleic Acids Res.* 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., *Nucleic Acids Res.* 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., *Strategies* 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc.,
20 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Phagemid pBS may be excised from the Lambda Zap and Uni-Zap XR vectors, and phagemid pBK may be excised from the Zap Express vector. Both phagemids may be transformed into *E. coli* strain XL-1 Blue, also available from Stratagene.

Vectors pSport1, pCMVSPORT 1.0, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained
25 from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, also available from Life Technologies. See, for instance, Gruber, C. E., et al., *Focus* 15:59- (1993). Vector lafmid BA (Bento Soares, Columbia University, New York, NY) contains an ampicillin resistance gene and can be transformed into *E. coli* strain XL-1 Blue. Vector pCR[®]2.1, which is available
30 from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. See, for instance, Clark, J. M., *Nuc. Acids Res.* 16:9677-9686 (1988) and Mead, D. et al., *Bio/Technology* 9: (1991).

The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID
35 NO:Y, and/or the deposited clone (ATCC Deposit No:Z). The corresponding gene can be isolated

in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species
5 homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X or the complement thereof, polypeptides encoded by genes corresponding to SEQ ID NO:X or the complement thereof, and/or the cDNA contained in ATCC
10 Deposit No:Z, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced
15 polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-
20 sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein
25 or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the polypeptides of the present invention in methods which are well known in the art.

30 The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or the cDNA sequence contained in ATCC Deposit No:Z. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X or a complement thereof, a polypeptide encoded by the cDNA contained in ATCC Deposit
35 No:Z, and/or the polypeptide sequence encoded by a nucleotide sequence in SEQ ID NO:B as

defined in column 6 of Table 1C. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X, a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z, and/or a polypeptide sequence encoded by a nucleotide sequence in SEQ ID NO:B as defined in column 6 of Table 1C are also encompassed by the invention. The present invention further encompasses a polynucleotide comprising, or alternatively consisting of, the complement of the nucleic acid sequence of SEQ ID NO:X, a nucleic acid sequence encoding a polypeptide encoded by the complement of the nucleic acid sequence of SEQ ID NO:X, and/or the cDNA contained in ATCC Deposit No:Z.

Moreover, representative examples of polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences delineated in Table 1C column 6, or any combination thereof. Additional, representative examples of polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the complementary strand(s) of the sequences delineated in Table 1C column 6, or any combination thereof. In further embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in Table 1C, column 6, and have a nucleic acid sequence which is different from that of the BAC fragment having the sequence disclosed in SEQ ID NO:B (see Table 1C, column 5). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in Table 1C, column 6, and have a nucleic acid sequence which is different from that published for the BAC clone identified as BAC ID NO:A (see Table 1C, column 4). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in Table 1C, column 6, and have a nucleic acid sequence which is different from that contained in the BAC clone identified as BAC ID NO:A (see Table 1C, column 4). Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides and polypeptides are also encompassed by the invention.

Further, representative examples of polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences delineated in column 6 of Table 1C which correspond to the same Clone ID (see Table 1C, column 1), or any combination thereof. Additional, representative examples of polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the complementary strand(s) of the sequences delineated in column 6 of Table 1C which correspond to the same Clone ID (see Table 1C, column 1), or any

combination thereof. In further embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in column 6 of Table 1C which correspond to the same Clone ID (see Table 1C, column 1) and have a nucleic acid sequence which is different from that of the BAC fragment having the sequence disclosed in SEQ ID NO:B (see Table 1C, column 5). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in column 6 of Table 1C which correspond to the same Clone ID (see Table 1C, column 1) and have a nucleic acid sequence which is different from that published for the BAC clone identified as BAC ID NO:A (see Table 1C, column 4). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in column 6 of Table 1C which correspond to the same Clone ID (see Table 1C, column 1) and have a nucleic acid sequence which is different from that contained in the BAC clone identified as BAC ID NO:A (see Table 1C, column 4). Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides and polypeptides are also encompassed by the invention.

Further, representative examples of polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences delineated in column 6 of Table 1C which correspond to the same contig sequence identifier SEQ ID NO:X (see Table 1C, column 2), or any combination thereof. Additional, representative examples of polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the complementary strand(s) of the sequences delineated in column 6 of Table 1C which correspond to the same contig sequence identifier SEQ ID NO:X (see Table 1C, column 2), or any combination thereof. In further embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in column 6 of Table 1C which correspond to the same contig sequence identifier SEQ ID NO:X (see Table 1C, column 2) and have a nucleic acid sequence which is different from that of the BAC fragment having the sequence disclosed in SEQ ID NO:B (see Table 1C, column 5). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in column 6 of Table 1C which correspond to the same contig sequence identifier SEQ ID NO:X (see Table 1C, column 2) and have a nucleic acid sequence which is different from that published for the BAC clone identified as BAC ID NO:A (see Table 1C, column 4). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in column 6 of Table 1C which correspond to the same contig sequence identifier SEQ

ID NO:X (see Table 1C, column 2) and have a nucleic acid sequence which is different from that contained in the BAC clone identified as BAC ID NO:A (See Table 1C, column 4). Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides and polypeptides are also encompassed by the invention.

Moreover, representative examples of polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences delineated in the same row of Table 1C column 6, or any combination thereof.

Additional, representative examples of polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the complementary strand(s) of the sequences delineated in the same row of Table 1C column 6, or any combination thereof. In preferred embodiments, the polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the complementary strand(s) of the sequences delineated in the same row of Table 1C column 6, wherein sequentially delineated sequences in the table (i.e. corresponding to those exons located closest to each other) are directly contiguous in a 5' to 3' orientation. In further embodiments, above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in the same row of Table 1C, column 6, and have a nucleic acid sequence which is different from that of the BAC fragment having the sequence disclosed in SEQ ID NO:B (see Table 1C, column 5). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in the same row of Table 1C, column 6, and have a nucleic acid sequence which is different from that published for the BAC clone identified as BAC ID NO:A (see Table 1C, column 4). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in the same row of Table 1C, column 6, and have a nucleic acid sequence which is different from that contained in the BAC clone identified as BAC ID NO:A (see Table 1C, column 4). Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention.

In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences delineated in column 6 of Table 1C, and the polynucleotide sequence of SEQ ID NO:X (e.g., as defined in Table 1C, column 2) or fragments or variants thereof. Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention.

In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences delineated in column 6 of Table 1C which correspond to the same Clone ID (see Table 1C, column 1), and the polynucleotide sequence of SEQ ID NO:X (e.g., as defined in Table 1A, Table 1B, or Table 1C) or fragments or variants thereof. In preferred embodiments, the delineated sequence(s) and polynucleotide sequence of SEQ ID NO:X correspond to the same Clone ID. Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention.

In further specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences delineated in the same row of column 6 of Table 1C, and the polynucleotide sequence of SEQ ID NO:X (e.g., as defined in Table 1A, Table 1B, or Table 1C) or fragments or variants thereof. In preferred embodiments, the delineated sequence(s) and polynucleotide sequence of SEQ ID NO:X correspond to the same row of column 6 of Table 1C. Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention.

In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1C and the 5' 10 polynucleotides of the sequence of SEQ ID NO:X are directly contiguous. Nucleic acids that hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1C and the 5' 10 polynucleotides of a fragment or variant of the sequence of SEQ ID NO:X are directly contiguous. Nucleic acids which hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described

polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence in which the 3' 10 polynucleotides of the sequence of SEQ ID NO:X and the 5' 10 polynucleotides of the sequence of one of the sequences delineated in column 6 of Table 1C are directly contiguous. Nucleic acids which hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence in which the 3' 10 polynucleotides of a fragment or variant of the sequence of SEQ ID NO:X and the 5' 10 polynucleotides of the sequence of one of the sequences delineated in column 6 of Table 1C are directly contiguous. Nucleic acids that hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides, are also encompassed by the invention.

In further specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1C and the 5' 10 polynucleotides of another sequence in column 6 are directly contiguous. Nucleic acids that hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1C and the 5' 10 polynucleotides of another sequence in column 6

corresponding to the same Clone ID (see Table 1C, column 1) are directly contiguous. Nucleic acids that hybridize to the complement of these 20 lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence in which the 3' 10 polynucleotides of one sequence in column 6 corresponding to the same contig sequence identifier SEQ ID NO:X (see Table 1C, column 2) are directly contiguous. Nucleic acids that hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In specific embodiments, polynucleotides of the invention comprise, or alternatively consist of a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1C and the 5' 10 polynucleotides of another sequence in column 6 corresponding to the same row are directly contiguous. In preferred embodiments, the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1C is directly contiguous with the 5' 10 polynucleotides of the next sequential exon delineated in Table 1C, column 6. Nucleic acids that hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

Table 3

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the

scope of the present invention. Accordingly, for each contig sequence (SEQ ID NO:X) listed in the fifth column of Table 1A and/or the fourth column of Table 1B, preferably excluded are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 and the final nucleotide minus 15 of SEQ ID NO:X, b is an integer of 15 to the final nucleotide of SEQ ID NO:X, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:X, and where b is greater than or equal to a + 14. More specifically, preferably excluded are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a and b are integers as defined in columns 4 and 5, respectively, of Table 3. In specific embodiments, the polynucleotides of the invention do not consist of at least one, two, three, four, five, ten, or more of the specific polynucleotide sequences referenced by the Genbank Accession No. as disclosed in column 6 of Table 3 (including for example, published sequence in connection with a particular BAC clone). In further embodiments, preferably excluded from the invention are the specific polynucleotide sequence(s) contained in the clones corresponding to at least one, two, three, four, five, ten, or more of the available material having the accession numbers identified in the sixth column of this Table (including for example, the actual sequence contained in an identified BAC clone). In no way is this listing meant to encompass all of the sequences that may be excluded by the general formula; it is just a representative example. All references available through these accessions are hereby incorporated by reference in their entirety.

Table 3

cDNA Clone ID	SEQ ID NO: X	Contig ID:	EST Disclaimer		Accession Numbers
			Range of a	Range of b	
HACBT91	11	789939	1 - 827	15 - 841	AW957974, AW003857, AI820045, AI523911, AW084836, AI983096, AI333432, AW188197, BF338099, N41047, AI433769, AI379333, AW438811, AA927750, BE139611, AI435307, BF436802, AW613586, R71675, AA678318, AA748856, AA301830, AI337423, AW243869, AA577365, AI382805, AI926335, AA568182, R47922, AA057770, R50081, BE247721, AI539624, AI698267, AI224055, BE244865, AI365443, N47137, AA903242, AV707146, AW955723, AV702790, AV701751, AW962407, AV705416, AI535639, AV705047, AW954994, AV703012, AW957544, BF380516, AV729076, AV707458, AV706060, AV704785, AV701728, AV709232, AW957628, AW954206, AV709235, AV707753, AV686390, AW955616, AW961393, AV725497, AV729376, AV728436, AV697196, AW958846, AW963660, AW951562, AW949999, AI535660, AV728733, AV692600, AV727314, AV703766, AV705319, AW959907, AW950888, AV656903, AW956199, AW957853, AV704180, AV696106, AV706223, AW963378, AW964540, AV727238, AV705869, AV705811, AW953969, AV689111, AW961593, AV695545, AV702266, AW952064, AV704955, AW963868, AV705135, AI525856, AW949731, AW958280, AV686420, AV686100, AV726103, AV695752, AV693523, AV709551, AV702861, AW951707, AV694674, AV709101, AV698429, AV702372, AV697498, AW950597, AW952223, AV692691, AV706229, AW958916, AV701067, AV658334, AV728670, AV705134, AV685966, AW955662, AI536138, AW956637, AV653325, AV705693, AW957102, AV705134, AV685966, AW955662, AI536138, AW956637, AV653325, AV705693, AV702498, AW950199, AV708203, AW964369, AV701643, AW950172, AV707002, AV725153, AV706136, AI557602, AW955904, AV727916, AW950411, AV707024, AW949529, AV706147, AW963601, AW953788, AV647006, AV702035, AV705520, AW954782, AW951743, AW957682, AV702172, AW966604, AV705453, AW952368, AW956762, AW962384, AV654287, AV708025, AV728309, AW950079, AV708980, AW965813, AW958901, AW959980, AW955841, AV705959, AV725697, AW950395, AV706910, AW959982, AW950443, AW950248, AV708850, AV707329, AV703635, AW954237, AV704798, AV703030, AV703542, AW959543, AW963011, AW963750, AW950671, AW955713, AV725948, AW949451, AW966756, AV729132, AV701881, AW949927, AV653809, AW955609, AV649672, AW950446, AW949351, AV702673, AW957110, AW954372, AV728874, AV685955, AW949530, AW959828, AW964111, AW960601, AV705185, AW966444, AW950012, AW962444, AV725001, AV703494, AW959806, AV705981, AW952403, AW957987, AW966603, AV726789, AV661704, AV706893, AW964673, AV727978, AV706459, AW953797, AV707414, AK025994.1, U94592.1, Y08991.1, Z30183.1, AF217994.1, U45328.1.
HADDE71	12	839187	1 - 653	15 - 667	BE646364, BE562975, BE734905, AA227916, BE275558, BE387443, AI568587, BE387535, AW245842, BE857544, AI805978, BE386863, AA530975, AA845548, BF437434, AW627607,

				<p> BE741623, BE898827, AA393921, AI201926, AI391625, AI199262, AI675180, AI123847, AA463396, AI128152, AI197839, BE791237, BE384118, AA913172, AA505110, AW408817, AI187762, AI076304, BE899199, RS2594, AW009600, AA465034, AA913634, AA488109, AA885156, AA452881, AA464960, AA884143, BF340639, AI886462, AI188491, BF446332, BF221728, AA227574, R84997, BE243531, BE903843, AA424231, BF331414, AI675231, BE249813, D45528, R40380, W60397, BE670322, F37062, R53393, AI473277, R84954, BE265829, AW407603, W60306, AA322573, AI928674, AI368380, AL535519, AI312011, AI886883, AI471250, BF032040, AA336279, AI357522, F31267, BE910005, BE311908, BE293522, AV681951, AI349772, BE964812, BG108147, BE047859, AW827203, BF054789, AV682330, AL513597, AL514803, AV682809, BG168696, AI868831, AW268253, AV682441, AL047042, AV682266, BF724691, BE047863, BF795712, BG058208, AV711509, AW071349, BE048071, AL514627, AL513907, AL513803, AV758592, AV723772, AL515041, BE613622, AV758110, AV710479, AV762488, BF673434, AV704928, AI349645, AV682249, AI815383, AV755581, AL135661, AV695052, BF968041, BE905408, BG033403, AV733397, AV682521, AV723204, BG179993, AI684265, AV756770, AI349614, AV682051, AV682772, AL119049, AV681668, AV682289, AV706777, AL514791, BG250190, AI207510, BF726322, AL514473, BE785905, BE880190, AV655645, BE881155, AV681630, AV682252, AL121270, AV723062, AL045500, AL513643, AV758668, AI906328, AV758217, AV681857, AV757012, BG108324, AV682082, AL120854, AV682479, AV681872, AL514935, AW080838, AV682074, BG105099, AV682466, AV729890, AV682222, BF981774, BG259801, AI349598, BF343172, AL515047, AL514155, BE048319, AV755613, AW467961, AV682672, AL515373, AI500553, AI907070, BG254754, AI064830, BE964700, AV733385, BF340104, BE777769, BE964486, AI687376, AW166645, AV682697, AV756703, AV682476, AV726951, AV756477, AV682351, BG109125, BG036846, BG259943, AL513631, AL515173, AI909666, AL514261, BG110283, AI340582, BE783707, AI436456, BF971016, BF969662, BE891101, BG178809, AV758806, AV755614, AV708119, AV757096, AL036396, AV681858, BE877769, BE018711, AW162071, BG114104, AV732941, AV723953, BE906959, AV710608, AV705644, AV733326, AV734318, BE048065, BF883916, AV681586, AL513763, AL514691, BG257535, AV704350, AV758738, AV729334, BF339420, AI580190, AI149592, AI345111, AI624859, AV682496, AL514919, BF344705, BE967113, AF078844.1, AF125949.1, AL512733.1, AL389978.1, S78214.1, AL133640.1, BC007021.1, AF090900.1, AF090934.1, AL442072.1, AL050393.1, AL049938.1, BC008387.1, AL157431.1, AB048953.1, AF090943.1, AB055303.1, BC008417.1, AL133016.1, AL110196.1, AB056809.1, AL136586.1, AL442082.1, AF090901.1, BC008365.1, AL050146.1, AL137527.1, AL117457.1, AJ242859.1, AK026608.1, AL136787.1, AL133606.1, AL117460.1, AB050510.1, AF218014.1, AL122050.1, AF104032.1, AB056420.1, AF090903.1, AK000212.1, BC008488.1, AL080060.1, AL390167.1, AL049452.1, AB049758.1, AL359596.1, AB063046.1, AL136749.1, AL110221.1, BC003687.1, AL359601.1, AF111847.1, BC003683.1, AL136789.1, AF106862.1, AK026865.1, AL162006.1, AK027868.1, AB047615.1, </p>
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HADDJ13	13	827273	1 - 2304	15 - 2318	AW575129, AW022897, AA010299, AU144131, AA669573, F11929, AL138228, AA634252, T66105, AA219059, R91924, R51726, AA856981, AA782322, AC079383.17, AC004552.1, Z95118.1, AF224669.1, AC018797.4, AC003035.1, AL161436.12, AC008625.5, AC002565.1, AC002350.1, AL390738.4, AC073593.13, AL354932.26, AC004491.1, AC011497.6, AC051619.7, AC008440.8, AF205588.1, AC007546.5, AC005250.1, AC016594.6, AC006038.2, AL031120.1, AC020658.6, Z84469.1, AC018719.4, AC006080.1, AF324890.1, AL354873.19, AL391259.15, AL162615.13, AL034372.33, AC018639.8, AL132777.4, AC004605.1, AL352978.6, AL160269.14, AL136000.4, AC024168.4, AL080275.20, AC090942.1, AC004685.1, AC008901.5, Z82198.2, AL121578.1, AC013734.4, AL133286.9, AC002091.1, AL356575.8, AC007934.7, AC005035.1, AL031311.1, AC005840.2, AL117337.25, AL035400.13, AC068724.7, AC007225.2, AL499604.9, AC005208.1, AL139785.5, AC002368.1, AC011484.4, AL162233.14, AC018637.3, AC091493.1, AL035555.10, AL136231.12, AP001714.1, AC044797.5, AC006211.1, AC009779.18, AL160155.19, AP000512.1, AC079754.4, AL391987.15, AP001732.1, AC083861.2, AC024060.5, AC025262.27, AL158069.16, AB023051.1, AC087311.22, AC078833.3, AC005670.1, AL391136.9, AL163267.2, AL080315.18,

					AC022201.4, AL138721.16, AC007318.4, AC005529.7, AL121594.6, AL136300.22, AL391114.12, AC006285.11, AL079342.17, AL031293.1, U82671.3, AC011310.3, U52111.2, AC018812.5, AL136365.9, AC078846.2, AL356379.10, AC007358.2, AL162231.20, AC000085.5, AC072061.8, AC010234.5, AC007151.2, AL445669.9, AL079340.7, AL445928.8, AC007363.3, Z94056.1, AC012377.5, AC006195.1, AL139109.14, AC002312.1, AC012368.6, AL022164.1, AC027124.4, AC005884.1, AC016574.6, AC010735.11, AL358815.12, AC008009.4, AL109964.20, AL161793.9, AL139092.12, AC010326.6, AC003950.1, AC002351.1, AC022468.5, AE000658.1, AC009484.3, AL031659.9, AC009247.12, AL138836.15, AL121595.5, AC004674.1, AC007386.3, AL355339.7, AC025168.7, AC021188.6, AP001731.1, AL138832.10, AC007228.1, AL355530.6, AJ009612.5, AP001671.1, AC015853.8, AC073607.19, AL137230.3, AL031295.1, AL122125.4, AC018641. 3.
HADMA77	14	783049	1 - 1899	15 - 1913	BE886986, AL118575, BF963505, BE896957, AA019981, AI871227, AW958870, AI148306, AI090074, AA594961, AI620245, N62115, AI499838, BE139496, AA253149, H37934, AW953772, AA398760, BE883579, AW008316, R17479, AI934670, AA253150, AI436173, AI446137, D59581, AI128007, R54297, H42535, D61056, D80139, R61184, AI197892, AA729655, D51488, H55864, H55771, AI075689, AA911710, AA292804, H86756, BF963502, T57670, AI961860, H42534, D51454, D81542, T34704, D80154, D81775, AA001440, T59256, D59479, R42975, R61185, AA994602, D51481, D51437, D59463, D59482, R46039, R51909, AA359928, AA659063, N79566, Z40268, R25021, R42826, D52193, Z42365, AA358058, T57710, AV656695, AL048093, R43779, AW276733, AA365016, AL048094, F01883, AA708838, C14366, N78346, AI601109, W16551, R18081, C00235, BF663261, BC007974. 1.
HADMB15	15	847116	1 - 316	15 - 330	AW136268, BG056888, AI131328, AI174443, AI091646, AW117296, AW168872, AI082447, AI432175, AI290911, AI741489, AI682685, AI142536, BG059892, AW149659, AW071935, AA233541, AI183690, BG056462, AI689641, AA599916, BF196591, BF196843, AA199743, AW136277, N77910, AA564806, AA243035, AA779709, AV722133, AI032138, AA844525, AI467910, AW965361, AA852418, AI982751, AI282445, AI982761, T03902, AI420648, AW167499, H08108, BE328548, AW068986, C15651, D52660, AW665899, AI246702, AI538705, AI271662, AI435112, AI288692, BE466948, AI690048, D55112, AA779042, AL536118, D53747, D54101, AA486941, D53384, W07076, AA232504, AA486765, BF832290, AI038647, AW497637, BF947006, AU155428, T05461, AL136582.1, BC001207.1, AB040527.1, AB058762.1, AB040528.1, AB040529. 1.
HAGBQ12	16	722205	1 - 729	15 - 743	AI332690, AI374724, AI285345, AA876359, AA987498, AI702600, AI079453, AI382918, C04098, R63800, AI697895, H87363, R67068, BE673734, R73892, BF432849, R68633, R68632, R66112, H03322, AA340294, H87907, AP000350.1, AC007363.3, AC003969.1, AC005948.14, AP000360.1, AC004066.1, AC000053.2, AC002060.3, AL450169.1, AL033522. 1.
HAGCC87	17	638587	1 - 1578	15 - 1592	AA587370, AA351604, AA661545, R22639, R22638, R18189, AA650370, AI459585, AI810301, R17880, BF434228, BE811111, BE701381, AA572974, BF857849, T50676, AL120282, AW168846, AI192440, AA714288, AI268019, AI745335, BF681619, BF828236, AU117483, BE147135,

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HAGHN57	18	773286	1 - 2426	15 - 2440	AL533248, AU118622, AU119331, AU133909, AU119469, AU118182, BE794468, BE791529, BG176702, BE280450, BE729801, BF663566, BF970116, BE257176, BG032912, AL516224, BG121097, BE784191, BG249033, BE727671, BE881192, BE745590, BF792305, BF037862, AV710149, BE617085, AV751361, AW291174, BG163346, AI686123, BG033409, AV762315, AV704873, BE540243, BF344980, AV707943, BF671351, BE394881, AW070780, BE538770, BF303671, BE541947, AW963773, BF303913, AW299817, BE378370, AW299807, BF107096, AW515893, AI338838, BE254836, AW402330, AA455894, AI436127, AL516223, BF001973, AI392820, W31025, W28207, BE535313, BE258523, BF109189, AA182513, BE617702, AW275883, AW674662, BG169977, BE711218, AA134574, AW304388, AA588768, BE868534, AU144819, AA455892, BF802948, BF222585, AW902162, HI6095, AI034153, AU145137, AI905391, AI985354, BG011776, AW612879, BE711276, AV659416, AU150558, BE702340, BF055535, BE711244, AA652292, AW271981, AA780056, AI624858, AA319693, AA604113, AV744893, AW771218, AV742941, AA837954, T60588, AA150957, AA151047, AI91761, AI912891, AI628783, AI434787, AW072744, AA716130, BF807693, AA181782, AI554969, AA916968, AA101864, AI473865, AA362607, AW338509, AI525459, BE244147, AI928082, AI433249, BF062859, AI910904, AA285264, BE711295, AI354885, AW006732, AI950274, AU144122, AI990867, AI922170, AA115829, AA806393, BE672240, AU156842, BE243206, AI633602, W01852, BE711219, AI280611, AA707161, AA301320, BF197637, AI695111, AW966603, BF447153, F29695, BE378061, AA336840, AI424341, AA385049, AI307649, N58884, AA131117, AI205138, BF431130, BF807685, N98771, AA602492, BE711204, BF438567, F34557, AA748737, T60437, AA745028, AW891490, AW673414, AI630237, AW378199, AW779341, BE172988, BE172375, AA101187, AA781579, AI478435, BE699167, R57333, AI927982, R92570, BE764834, BF818234, AA648053, BE464290, AK000994.1, AC004668.1, AL050216.1, AA227675.
HAGHR18	19	655435	1 - 1128	15 - 1142	AA678513.
HAQA192	20	688037	1 - 593	15 - 607	AL522436, AL524148, AA513002, AI735602, AA772397, AW014080, AI799589, AI818675, BE617237, AA478326, AI217776, AW409592, BE646171, AI005409, AA552147, AW177019, H42123, R00846, H42122, BE795741, AA362478, AI491808, AI769438, AI560335, BE613582, AI336126, AI371050, R01499, AI610208, BF089287, BF095098, AW613379, AI275309, BF752321, BC004222.1, AL118502. 38.
HAQBG57	21	837545	1 - 1034	15 - 1048	BE562515, AI742455, AI961996, AA507216, AI190639, AA731364, AI673081, AA593594, AI003558, BF528073, BF885284, AI435334, AW438908, AI381200, R69327, AI204170, AI739035, AI401755, AI591140, AA432147, AA251459, AW967618, AA446655, AI682154, AW613695, AI500259, AA398391, R79970, AI149747, H97579, AI263813, AA401707, BE350370, R69328,

HAQCE11	22	633730	1 - 582	15 - 596	AA670245, AA643922, AA852081, BF345349, AA889222, BE782687, BF034010, BF038771, BC008671. 1. N45328, H29603, F10900, AW949645, D80045, AW965158, AW949642, AV738340, AV742732, AV724520, AW949643, AV741220, AW964468, AW966389, AV718489, AW966330, AV699550, D58283, AV718692, AW975618, AV742048, AW978634, D81030, AV718844, AW959570, AV720203, D80043, D59619, D80195, D80210, D80240, D51423, D51799, D80391, D80253, AV719822, D80227, AV719324, AV719783, AV718800, AW966531, D80188, AV720211, AV720464, AV718770, AV720731, D80219, AV699447, AV722801, D80196, AV723927, AV699927, C14429, D59927, D80038, D80212, D80193, D80022, AV719468, AW949632, F13647, AW949641, D80366, D59889, AV700889, AV720812, AV721386, AV723097, AW973447, AW949656, C15076, AW949629, AW966062, D59275, AW966053, AW964532, AV701335, AV742001, AV701043, AV701332, AV701017, AV701248, AW959628, AV701431, AW949653, D80378, T03269, AW949633, AW949658, C75259, AV744690, D50995, AW949657, AV719188, AV719557, AW949631, AV742667, AV701125, AV701166, AV700229, AV699746, C14014, AW973307, AW975621, AV702035, D80134, AW949646, AV701419, AV699479, AV701154, D80164, AW959582, AV701443, AV744012, AV720028, D59502, D81026, AV719628, AV718707, D80166, AW966534, AV701130, AV701149, AV701422, D59859, D80268, AV718440, AW965177, AV741221, AW949618, AV645344, D51250, D80269, AW960553, AW966054, D80168, D59467, AV701428, D80949, AV701415, AV701344, AV719000, D59787, AV745080, AV645389, D80522, AV681510, AV681491, AV701021, AV645343, AW959202, AW960414, AV681505, AV681504, AW949654, AV721784, AW973541, AW966013, AV720654, D58253, AW966041, AV719913, AV718674, AV705134, C14227, AW959597, AW964737, C14331, AV720791, AV701004, AW978661, AW973470, D50979, AW966050, AW752082, AV720220, AV700622, AV699669, D80024, AV743601, AV681529, AW966043, AV704180, AV705869, AV706229, AV701224, AW949655, AV719049, AV701330, AV681468, AV720607, AW960465, AW973334, AW964488, AV681474, AV741012, D57483, AV742671, AW973485, AV744773, AV718633, AW975605, AV681526, AV727978, D59610, A1905856, AV699682, AV718931, AV744934, AV645383, AV645393, AW959799, AV681528, AV645339, AV681472, AW978648, AV681507, AV681465, AV681525, AV681495, AV681486, AV681464, AV681487, AV681506, AV681523, AV742720, AW966029, AV742430, AW966022, AV738928, AV726812, AW966023, AA285331, AV706147, D59695, D81111, AV744768, AW949620, D80241, AW958992, AW964756, AV681514, AV681477, AV681512, AV681497, AV681483, AF035294.1, AB028947.1, AF271371.1, D34614.1, X67155.2, D88547.1, AF058696.1, AB028859.1, AB002449.1, U79457.1, Y08991.1, U94592.1, Z30183.1, D50010.1, AB033111.1, S78798.1, X92518.1, AB038216.1, X98248.2, U45328.1, X60736.1, AF217994.1, AB035274. 1.
HBAGD86	23	838799	1 - 1699	15 - 1713	A1658681, BE466145, A1806836, A1653272, AA004211, BE302094, BF970406, BE018485, AA418617, AA594901, A1580148, BF589715, A1804211, A1669907, A1342168, A1810310,

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HBGBC29	24	691473	1 - 1842	15 - 1856		BF223021, BF036281, AI341667, AA180986, AU153625, AU151704, AI093197, BE855464, BE018834, BE616741, BF684563, AI694268, AA031711, AI469856, N63041, N50125, AI150599, AI597740, AI985206, AI671591, W72535, BF431270, AI741942, AA037642, AA180865, AA031648, AA436065, AI800796, AA129939, BF056140, AW022665, AU157670, AI074205, AA830493, BF063800, AI056532, AI656721, W00519, AI275143, AI337739, AW172525, AA443349, AA043021, AA446926, AI655558, AI769027, AA101851, AA917703, W93307, AA526333, AI689128, AA777090, AW002829, BE295568, AW139517, AI128702, AI276137, AW801873, AA873711, AW892754, N98234, W76109, AI631104, AA856832, W92810, AA042939, H87505, AA129938, AI688779, AA693329, AI676108, T87624, AA570072, AA037641, AI186390, AW515672, AA031685, AA037500, R82703, AA037234, AW380430, AA985191, AU131994, BE302396, H87506, AA938640, AI926907, AU118291, AI696069, T74071, AA102060, AW057528, AI671894, AI962374, AI695458, AA046964, BE869607, BF814627, F12449, AA725452, AI968837, AA917824, AA054749, BF437316, F10070, AA917678, BE218382, BE669660, AI916503, AW612381, AA683581, AI984598, AA937814, AI932475, AA046963, AA053281, AI801723, BE858841, AI499751, AA031686, AI074981, AI341558, AI478279, BF735972, AK001006.1, BC004523.1, AF020920.1, AF038662.1, AB024436.1, AF022367.1.
HBJAB02	25	837309	1 - 1679	15 - 1693		AL529646, AL529645, BE898304, BG112747, BF791411, BG036058, BE392384, BE621757, BE548173, BE895853, BG034671, AA808894, BE901085, BE278873, AW152607, BE795658, AW166898, BG122141, BE782474, BF972826, BE793716, BE140314, AW750993, AA826362, AW517942, BE714673, T59668, BE731030, BF939314, BE732766, BE745104, AI290469, BF477770, AI805651, AI961329, AA581089, BE902575, AW197375, AA974066, AI950259, BF802171, W27729, AV693783, AA877530, AA715365, AI968889, AA885542, AA160748, AA386371, AA335719, BF873961, W73105, BF223151, BE740826, AL120854, BE548914, AA318192, AA501478, BF125073, AI948815, AA581100, AA658457, AI621069, T59802, AA468534, AA503715, BF850755, AW956069, AW841506, AI144504, AA352215, BE897964, BF883404, BF373009, BE090290, BE168997, AW855521, AW820855, BG230749, BF376598, BE622839, AV699089, AV647789, AI567702, AV726156, AW961037, AW411235, AV726058, AW020397, AV706279, AV702427, AV651955, AV702026, BE393551, AV727787, AV660608, AV687176, AW021717, AV698545, AV687909, AV709256, AV708438, AV656903, AV661704, AV696106, AV697196, AW409775, AW951263, AV689111, AV655280, AV728157, AV692345, AV659322, AV654908, AV656478, AV708893, AV709314, AV708381, AV660728, BG168549, AV659536, AV691080, AV706219, AV695545, AV652001, AV705159, AV648263, AV703169, AV728518, AV707541, AW952409, AV709660, AV726624, AV706854, AV729220, AV709604, AV687035,

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HBMUH74	26	866160	1 - 712	15 - 726	AI633540, BE999936, AL529110, AI911597, AW016785, AA479308, AI381011, AI057451, AI283542, AI224172, AI025510, BF929951, AW589256, AU156824, AU155569, BF063133, R43074, R25758, BF818086, AL529111, BE567017, BE077233, H09061, AA479409, AL136843.1, AK001927.1, AK027756.1, AK001324.1, AC009318. 11.
HBNAX40	27	834801	1 - 2779	15 - 2793	BF966078, BF792338, BF034911, BF217973, BE883387, BF947401, BF574197, BF060683, BE220005, BE645102, AI808818, AU158323, BE222311, BE467629, BF985268, AA203305, BE504175, BE612371, BE504478, AI890286, BF514573, AW173142, AI674096, BE301797, AW962903, AI674111, AI935063, AW958697, BE931820, AI431629, AI418384, AU157624, AW958686, H10461, AW995348, AW511978, AA864829, N29528, AI287632, AU157306, AW206871, AI381961, BE018315, AW238878, R61198, H80193, AA531283, AA565321, AW073280, AW026572, AW243789, AI819460, AI913516, BE503173, AA305587, AA305897, AW952734, BE815642, C01749, H10462, R58932, AI040839, T17118, R62976, H17748, H11865, R63031, AA970617, AI247608, R63338, T07723, AA774553, AA371079, BF084654, AA329699, BF802622, AW139568, AW027833, R61199, H11505, N57467, R19912, BE695240, R63337, R06902, AA337757, R33944, R14158, T55659, AA247547, AU140145, AA329948, AI872648, H17635, T55578, AI802966, N67275, R40453, AA318909, AA039614, AI565257, AI540752, AW865932, N46626, R06946, N40466, T25153, AI444969, AI262121, H78817, AW150803, AI003014, AI457343, N34900, AK023746.1, AF264912.1, AF305081.1, AF329696.1, AF342982.1, AL356305. 11.
HBXCX15	28	637542	1 - 1205	15 - 1219	AA595781, AW277007, AI274544, AA548746, AC006329.5, AC009412. 6.
HCDBO32	29	831942	1 - 2616	15 - 2630	AL537440, AL531001, AL531000, AU118775, BE613081, AW976126, BE739778, BE612415, BE886668, AW965087, BF671603, BF114976, BF001395, BF104843, BG169691, BF790959, AU145261, AI524826, AV700940, BF115561, AI628083, BE501914, BF028814, BG107506, AA456561, BF695399, BF064237, AW771567, AI554053, AU152051, AI066556, AI478798, BE962627, AW770611, AW963335, BE739003, BF064238, AI801476, AI807830, AA913477, AI424225, AA227589, BF575758, AA625584, AI963182, AW771337, AA576069, AI252762, AA070604, BF589915, AV702872, AA428503, AA235962, AI539101, AA419520, AA721024, BF700344, AI357722, AA314319, AA310761, AA235961, AA886887, AA479915, AW300423,

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HCEEE79	30	560609	1 - 1038	15 - 1052	AL353658.33.
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HCUCF89	32	637986	1 - 516	15 - 530	<p>BF909758, BC001698.1, AF225418.1, AL133067.1, AL049300.1, AL110196.1, AB055368.1, AL122050.1, AL096744.1, AF090934.1, AL137529.1, AK025958.1, BC006201.1, U42766.1, AL133565.1, AF218031.1, AL136640.1, AK026784.1, AL117457.1, AK026434.1, BC007199.1, AL136787.1, AL389935.1, AL162006.1, S61953.1, AB060908.1, AL136790.1, AF097996.1, BC007926.1, AB062978.1, AF218014.1, AF090900.1, AB047631.1, AB047941.1, AL512733.1, AL050393.1, S78214.1, AL357195.1, AB060929.1, AL133557.1, BC004908.1, AB055303.1, AB060887.1, AF207829.1, AL133080.1, AL133640.1, AF210052.1, BC000090.1, AL359601.1, BC003120.1, AK026518.1, AL137459.1, BC001293.1, AK025015.1, AL035458.35, AF078844.1, AL050116.1, BC003683.1, BC008417.1, AB052200.1, AL442072.1, AK027868.1, AB060229.1, AK026353.1, AB060873.1, AL512746.1, AF111847.1, AL133606.1, AK026542.1, AB056427.1, AK024992.1, BC001967.1, BC004370.1, AL049314.1, AB060837.1, AL050146.1, AL442082.1, AK026506.1, AL137527.1, AB049892.1, AF022813.1, AL049382.1, AB063008.1, AK027161.1, AL359583.1, AK026741.1, AB048974.1, BC006525.1, AK026927.1, AL157431.1, AL162085.1, AF090896.1, AL122121.1, AL162002.1, AK026583.1, AL049430.1, AK026797.1, Z37987.1, AL136789.1, AL133016.1, AK000323.1, AL122118.1, BC006807.1, AL136749.1, AB055361.1, AL137283.1, Y16645.1, AF090943.1, AK026480.1, AF285836.1, AK026522.1, AK026630.1, AK025708.1, AK025484.1, BC008387.1, AB060826.1, AK026086.1, AL133093.1, AB063070.1, AK000432.1, AK025339.1, BC004195.1, AF146568.1, AL137705.1, AL122123.1, BC003104.1, AK000618.1, BC008836.1, AK026855.1, AL136844.1, AB056420.1, AB052191.1, AK025092.1, AL049452.1, AL117460.1, AF090886.1, AF090903.1, AL136845.1, AK024538.1, AL137292.1, AL136799.1, AL512719.1, AB047615.1, AK026744.1, AB056421.1, AL117583.1, AL442083.1, AK024974.1, AK000212.1, AK000083.1, AB056809.1, AL110221.1, BC007680.1, AK026452.1, AB047904.1, AL353956.1, AF056191.1, BC006195.1, AL136784.1, AF104032.1, AF091084.1, AL512750.1, AL512718.1, AK026045.1, AB060863.1, AF125949.1, AL137488.1, AL390167.1, AL080124.1, AL137276.1, AL050277.1, AB048953.1, AL512761.1, AL137271.1, AL359596.1, AB063046.1, AB055366.1, AF120268.1, AJ242859.1, AK025632.1, AL080127.1, AB049758.1, AK026924.1, AL137550.1, U72621.3, BC001418.2, AK024594.1, AB051158.1, BC006440.1, AL049283.1, Y10936.1, AK025375.1, AK025414.1, AK025573.1, AB060852.1, AL512689.1, AL122100.1, AL583915.1, AL110225.1, AL136892.1, AY034001.1, AK026600.1, D83032.1, BC007021.1, BC003687.1, AB047878.1, AL136884.1, BC008488.1, Y14040.1, BC002839.1, AL389983.1, BC008899.1, AK000137.1, AL049466.1, AK026959.1, AB048954.1, AB063088.1, AL136893.1, BC008282.1, BC004119.1, AL136586.1, AF090901.1, AL122093.1, BC008893.1, AB060214.1, AL096751.1, BC008365.1, AL080060.1, AK027113.1, AB060912.1, AL162083.1, AL136767.1, AL049938.1, AL512684.1, BC002647.1, AK000445.1, BC006408.1, AF069506.1, AK025084.1, BC007556.1, AL133075.1, BC003548.1, AL137533.1, BC008078.1, AL136864.1, AL137548.1, AF106862.1, BC006832.1, AK026591.1, AL133560.1, AK000652.1.</p> <p>AL1524118, BE277210, AL039145, BF698704, BE276480, BE409047, BF698510, BG150796,</p>
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HCWAE64	33	535893	1 - 457	15 - 471	AL043265, BE895962, BF091850, BF924502, BF930204, AW973724, BE906549, BF972009, AA558125, BG163769, AW993087.
HCWUL09	34	834722	1 - 747	15 - 761	AL138741.13.
HDPDI72	35	897277	1 - 1536	15 - 1550	AV717810, AC018828.3, AC011464.5, AC022383.3, AC022384.4, AC034193.4, AC002472.6, AC021015.4, AC008119.6, AL356299.16, AC004951.5, AC018808.4, AF003626.1, AP000215. 1.
HDPFY18	36	779450	1 - 2173	15 - 2187	AW792967, R41077, BF921165, AV750453, AA729108, AA715505, AW975570, AA714451, BF826980, AW999989.
HDPIE44	37	899328	1 - 4101	15 - 4115	AL528314, AU130887, BE780963, BF966718, AL118570, BE739397, AU126476, AU139315, BF512830, BG115027, BE880693, AI908306, BF791388, BF035925, BF540985, AU122664, AV724420, AI672418, BF665031, BF057158, BF206660, BG120429, BE738855, BF129883, AW959181, BF106186, BF966523, BF213412, AI042351, BF211302, AL042137, BF515714, BF206917, BF031857, BF131974, BF129874, BE514139, AV696443, BF037314, AV661523, AW130577, AA424461, AW130565, AA902916, AU127429, BF677116, AU150429, BF665735, AA983275, AI239435, BE155330, AV705068, BF212900, AV710135, BF432386, BF057834, BF211687, AA461260, AA312396, AI627321, AI160503, N63373, BE502195, AV751301, AI884925, BE468064, BF513143, AV750990, AA186321, BF184200, BE559625, AA749086, AW025721, AU152756, AI418879, AA402517, N26419, AA088855, AI042350, BE268734, AI761107, AI819134, AA678020, AU150014, AA113363, AW341372, BF184441, AI457727, AA649198, BE674349, AA888970, AA580858, AA199866, BE270920, AV649723, H24854, AA100701, AW025691, BE836719, BG010680, BE550985, BE087003, AA326555, R77878, AA424417, AA188173, AL044709, H05951, BF792719, BF930036, N56631, N41679, BF923482, H45855, AI218982, AW779309, AV751002, BE089649, C17343, BF081703, R81295, AA502426, BE646243, BE931964, AW594429, BE676875, R11716, BF748564, H06000, AA460953, BE832883, AV703744, AI265945, AV751157, BE720186, AW021533, T09412, BF849176, AA088766, AA437111, AA336899, BE262777, AA358792, BE155518, H99315, BG166471, BE270762, BE313275, BF239379, AA888732, BE825194, T89695, D78877, AI269641, BG011279, AI469067, AW874055, AW338714, AW874611, N88500, BF445639, AW058314, BE677169, AW139207, R17691, T31819, AW513547, AW472793, AA358708, AV660383, AI765491, AI582998, AI084965, AA598573, AV750235,

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HDPIU94	38	813352	1 - 2182	15 - 2196	AU140297, AL529544, AL529545, AU124978, AI740820, AU116885, AU126162, AW960772, AI565169, BF111956, BG251247, BG177689, BE780814, AI628285, AA482031, BE784432, AA947029, AW954823, AW190175, AA315300, AU143854, AA707674, AI332610, N50136, AU148736, AU127152, AW768480, BF947113, AA223261, AW955931, AI276839, AA189165, AA804584, AA767472, AA223378, AA894857, AA252718, R46372, AA939277, N59367, AA219127, AA774827, AV762911, BE546354, N72682, AA219510, AV761697, AA872005, AW188325, W02461, R21326, AI923716, D29223, R68368, BF771937, BE769443, AA322537, R08745, AA417592, R08746, AW952240, AA299861, AW377015, AA337351, H60482, N76470, BF088734, AA218745, AA336556, BE896274, AI810734, AW118290, BF380800, T30177, D29202, BF910258, AA337527, AA336555, R68574, AI167609, AA376922, AW968355, BF351657, AI832198, AW972092, AW968356, AW972093, AW968729, AI432644, AI623302, AW971740, AI432654, AI432650, AI432653, AW081103, AW858522, AW972091, AW969229, BE72759, AW972090, AI432677, AI431230, AI431307, AI431316, AI431328, AI431353, AI431312, AI432655, AI431310, AW128900, AI431238, AL045327, AI431354, AI432666, AA580821, AI431347, AI431315, BF448552, BE672748, AI432661, AL134524, AI431323, AI431337, AI432675, AI431321, AI492519, BE672745, BE672732, AI431246, BE672719, U46344, AI431235, AI431243, AI432647, AI432651, BE672738, AI431255, AI432674, AI431330, AI432649, BE672767, AI791349, AW601637, AI431248, AI431241, AL042842, AI431254, BE672774, AI431357, AL042729, AI432672, AI432665, BE672742, AL042931, BF589777, AI432662, BE672627, AW577201, AI431345, BE672644, AL042655, AI431351, AL042508, AI431231, AI431346, AL042853, AI432676, AI432673, AI432658, AW128884, AI431257, AW577199, AL042533, AL043166, AL047611, AI431340, AL135012, BE672622, BE672792, AI432657, AL042802, AW128846, AI431247, AI432664, AI432645, BE672718, AL042787, AL042515, AL042832, AI431751, AL043295, AI431314, AI492520, BE672634, BE672743, AI355008, AI492510, AL042898, AI431350, AL043091, AI431318, BE883591, BG167830, BE672749, BE672744, AI682915, AL040207, AW128897, AI866786, AW129223, AL042488, AI432643, AL043278, AK022626.1, BC001240.1, AK001284.1, AF064854.1, AL133074.1, AL133053.1, AL136763.1, AL133049.1, AL133076.1, AL122101.1, AL136755.1, AL136758.1, AL133068.1, AL136825.1, AL133051.1.

HDPDPD93	39	637588	1 - 687	15 - 701	AI767544, BF963878, AW391604, AW371053, AW391605, AW380560, BE150935, AW380557, AW609397, AV647627, AW609527, AV647628, BE150882, AA625481, AV647780, AW582425, AW582423, AA053357, Z39028, T31703, AI559952, AI962812, AW023035, AW247321, AW594578, AW204377, AW135110, AW069029, AA737065, R51075, AW294433, AW291912, BF511819, AW292027, AI265783, BF063801, AI274716, AI191509, AA605269, AI768872, BF057534, AI634812, AI698531, AA565825, AI275563, AI366661, BE242780, AI538787, AI203337, AI565080, AW008059, AW088320, AW073352, W73510, AW242351, AI341846, BF222967, AA513453, AI346467, AA622940, AI000935, AA535424, AI022694, BF592958, AA639759, AI679288, AI679864, AI079193, AU147451, N56986, AU148991, AI304785, AA134162, AW664542, AI337342, AI245649, AI969305, AW582354, AI081970, AI262353, AL079933, AW976197, AI991639, BF349821, AU159502, AW004865, AA665733, AI348128, AI094163, AI184073, AU152120, AU149126, AI359191, BE552234, AA632288, AA814449, AW362622, AU159545, N75380, N49596, BF869959, H48051, N70679, BF987335, AI864296, AA928727, BE963530, AA617935, AI151119, BF680667, AW978110, BE785995, BF913501, AL519088, H56669, BF526020, AI668893, AV655645, AV714752, AW087445, AW104724, AI538716, AL045500, AI564719, AI802542, BG058398, AW026882, BG031815, AI433157, BG179993, BF812961, AI620284, AI524671, AI857296, AI619502, AW118557, AI682841, AI439745, AI934035, AL514129, AL036274, AL036361, BG111590, AI560099, BE048071, BG260037, AI445432, AL036802, BG257535, AV732936, BF970449, BF812938, BG110517, AA640779, AI536685, AI445025, BG180996, AL514983, AL121365, AI521012, AI537677, BE884130, AI475371, BG112718, BG113299, AI702406, BG110684, AI349004, AI580984, AI687127, AL513907, BF032868, AW166970, AI687375, AI625079, AI440239, AI349645, AI635461, AL119049, AI433976, AW071349, AV753074, AV757018, AI285735, AV758209, BG109270, AI271786, BF792961, AI498579, BF882343, AI281762, AI690480, AI815855, BG170430, AI783504, AL043975, AI636456, AI687728, AV682093, AV708097, AW302988, AI432969, AW148716, AI800384, BG112879, BF924882, AI697137, AL514879, AL036396, AL048656, AI500077, AW827211, AI282655, AI962456, AW268253, AV681618, AV756619, AW086113, BF793244, AW129659, BF885675, AV758822, BF816811, AV758592, AI799470, AI539780, AI828682, AV681869, AI610307, AI612913, AW132056, AI872711, AI818683, AI499393, AI493248, BE887488, AV713079, AV652906, AI340519, AI537303, AW071417, AI475451, AL040243, BF107577, AW082040, AL036146, BF038106, BG036846, BF726297, AV682849, AK026796.1, AK027770.1, AL391244.11, AF251294.1, AL136882.1, AK022241.1, AB051496.1, AK026647.1, BC008387.1, BC006195.1, AF090903.1, AB055361.1, AL117394.1, BC008488.1, AB060826.1, AB063070.1, S78214.1, AL117457.1, AK025339.1, AK025092.1, AF125948.1, AB060912.1, AL359615.1, AL133606.1, AL122050.1, AL049452.1, AL157431.1, BC001967.1, AL049314.1, AL162006.1, AL137459.1, AL512746.1, AL050149.1, AK025958.1, AB055303.1, AB060887.1, AL050116.1, AL110225.1, AB048953.1, AB056420.1, AL050277.1, AL512733.1, BC008365.1,
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HDTLM18	40	836057	1 - 511	15 - 525	T62863, AL049843. 18.
HE6CS65	41	762960	1 - 1512	15 - 1526	<p>BG114804, AV718161, BG115294, BG163956, AW362005, AW579708, AA425593, AV732860, AA778426, AW367244, AL537244, AW856936, BF377273, BE675130, BG119784, BF978611, AA194252, AI937228, AW292921, AI222740, BF349929, AW856088, AI348188, AW665835, AA025880, BF333804, AW005582, AI139606, AI126585, AW959277, AI417243, AI339985, AI972128, W52543, AA829354, BF333819, AI078819, AV752850, AW857034, BE789386, AI299395, AW675446, AI083537, AW516855, AA035526, AW005637, AA932845, AI088259,</p>

					AI492529, BF375157, AI334135, AI682947, AI753237, AI923621, AA425629, BF804458, AI628857, AI635633, AI085676, AI580195, AI138968, BE350523, AA005425, BF333807, AW102884, W52544, AI022376, W39677, AI920867, BF333811, AI240384, N49885, AA227905, AI087384, AI302240, AI933336, AI670129, N98508, AI268859, AA232843, AI221780, AA253379, N71409, AA004925, BF761279, AI066394, BF811217, AI302764, AA311893, AW571609, AI080166, AI299456, AA844294, AI342664, AI147714, AA464423, T60255, BF807121, AA765193, H29427, AA876118, AW265722, AI074827, AA808646, AA193122, H43349, AA233282, AI693779, AI245406, R60024, AA005426, AA995705, AW302271, AA934521, AI061151, AW366962, H38317, T90537, AI445689, W44818, H12893, AI446122, AA253378, H78422, AW378644, AI753445, R44689, N99734, AW102871, AA719170, AA464422, AA974340, H78222, AA934622, AW149309, R68103, AW265649, H40537, AA807276, H43181, AI277059, T34512, AA430350, BE539364, AI076849, AA347001, T81330, BE049268, H26609, AW589372, BG231066, AI092386, R24873, R43362, Z40090, AA334085, AW378680, N31595, H58243, R68147, AA257048, AA004926, H12894, AA886683, BF593823, F02035, AI559907, AW062917, N49990, H43311, AA295950, AI364249, R52133, BF748425, BF804455, AA778257, AW379516, T81506, AI080028, AA347002, BF377711, T83072, AL134712, H26654, BE082628, BF131565, AA227588, BF092102, BE774491, BF092109, AI382725, W63710, BF807108, F01928, BF858654, W31073, AA169731, AW367337, AA936648, AA193460, AW378675, H43212, BE082824, BF858661, AW864790, AA946635, T61637, AA304629, R17644, BE047631, AI867081, T24808, AA293625, R52222, AW890658, AA425423, BF929843, BE88442, C02346, Z99943.1, AK024700. 1.
HE8BQ49	42	589443	1 - 1861	15 - 1875	W35257, AC009225.3, U49973.1, AF036938.1, AL445225.9, AC006142.1, AC006077.1, AC008670.4, Z74739.1, AL023755.5, AC008780.6, AL445212.9, AL356317.8, AL137244.28, AJ271735.1, U70984.1, AF241734.1, AC021506.5, AL391868.15, AL162386.17, AC009312. 4.
HE9CY05	43	834826	1 - 1033	15 - 1047	N76568, N54458, H74303, H74302, H73373, R02548, H40263, H58326, AI242058, H58715, H73374, R02666, AI438986, T80187, AA676653, AI022453, T87491, N86939, N88474.
HEAAW94	44	847340	1 - 910	15 - 924	AA578368, AA492135, BE707438, AI880112, AA927112, AW970866, AA368674, AW237957, BE560582, BE560540, BE268041, BE513979, BE398109, AI525375, BE270770, AF191611.1, AF043045.1, AF042166. 1.
HEBFR46	45	847064	1 - 1290	15 - 1304	BF339246, AW957665, BG258103, AW075995, BF309372, BE868083, AW576203, BF308177, BE881903, BF689190, AI051657, AA311371, BG059809, W56301, AW058408, AA102223, BE301190, AI091799, R05745, D61582, R01123, AA102222, AA375163, BG029189, AW293550, AI752483, AA376452, AW275432, BF812696, AI439525, AW151541, AW084324, AL121039, AW265468, AI702049, AW162314, AW327673, AA577706, BE273825, BF940118, AI270280, AW148821, AW162332, AA807704, BG059139, AA661583, AW238137, AA601674, BG180320, AV742390, BE244308, AW410844, AI433952, AI828721, AA631915, AL079734, BG152746, AW473160, AW021399, AW020094, BE677164, AA728954, AI860423, AI039257, BF679568, AW243817, AI049999, AW148964, AI538404, AI826857, AI753131, AI690379, BE676856,

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HEOMC46	46	866171	1 - 925	15 - 939	AC008857.5, AC007845.12, AC005091.1, AL132713.11, L78810. 1. AW026120, BF891831, AI498747, AI056326, AI075298, AI359561, BF901563, BF901553, BF893716, BF894749, BF901561, AI457604, BF891852, BF901564, BF901551, BF893717.
HFCDW95	47	847383	1 - 857	15 - 871	AL529530, AV726582, BG180774, AW952054, AA398982, AL537902, BE739764, AV727582, AW300512, AV722244, AW029553, AI986473, AI950933, BG164817, AV726968, BF588526, BF476107, AW770808, BE874188, AA639868, BG252620, AI978599, AA142949, AI700677, BG059521, AI918056, BE866188, BE738987, AI828361, BF185676, BF445290, AA399621, BF031768, BF697098, BE785930, BF433181, AI380426, AU150075, BF030153, AI380761, AI040457, AW302413, AI678823, AA737313, AA548083, BE905006, AI273446, AI632020, BF001920, BG151519, AI887157, BF446900, AI925691, AI304432, AI375004, AA814501, AI284941, AI819675, AA708445, AW131704, AI478462, AL529529, AI741247, AA969450, AI308781, AA136378, BG054885, BG258115, BF701370, AI680947, AW148776, AV727838, AW304864, BF208666, AA432085, AA279397, BF028795, BF028097, NS2155, BF031629, D54791, BF591720, AA809906, AA155617, BF028402, AI803830, AI347883, AA983660, AA604572, AI262096, AW236261, AW576520, AA157854, AA588204, BF131266, AA088711, AA282014, AA702285, AA923508, BE048565, BF667411, AW780109, AI262793, BF341242, AA548251, AI421476, BF939796, AW194950, AW337256, D56471, D52957, AW887069, AI183568, HI7142, BE565940, AA137224, D52438, AA771875, D58681, AA137223, AV725549, AA150656, AA911258, AA446770, D54998, R79409, H09565, R33682, BF207904, AI613214, AA658190, AI421135, BF091420, F00903, BE184725, BE184795, D53702, BF448933, AI421134, AI080427, AI612899, M78614, BF028440, F00618, F04716, BE549554, AI816921, D51012, T35003, AI301723, AA484869, AA335305, BE866601, AI498636, H88563, AW801803, D52945, BF222159, AW801965, AA514008, H22397, AI424765, AI885113, BE896219, AI365192, T05851, R77482, AA652212, AA255908, AW134716, BF890688, N62323, T07892, T36093, BF833829, T31797, BE843694, T32233, F00108, AI811601, T30493, BG259677, D56534, F00210, R35636, M78491, N41045, AA995329, BF967013, BE926012, F02051, AA513039, BE865924, BE539516, BF476761, AI890228, BE184726, AW837831, AW607908, BF840954, D55509, BE811512, AW003098, BE891332, BE888598, AW750879, AW630713, BG104880, BF038524, AA136287, AA320122, AW138663, AW627379, AW028138, AA828915, BE702523, AA256021, BF131079, AW236903, R35739, T95736, AA912070, AV726830, AW897704, AA629231, AW272373, AW798454, AI269580, AA857847, AW075519, AI783861, AI367203, AI624293, BF885000, BF814450, AI611743, AI670009, AI560679, BF970652, AI282355, AL513755, AI333104, BE966990, AI868204, AL514511, AW089844, AI554821, AL514765, AI799674, AW058207, AC006388.3, AB024334.1, AK024230.1, AL117438.1, L30117.1, AF217987.1, AL117648.1, AL136925.1, BC005805.1, AL136893.1, AK026927.1, AL080129.1, BC001045.1, AF090900.1, AB052191.1, AL512750.1, AL512719.1, AK026164.1, AL389947.1, BC006525.1, BC002409.1, BC004310.1, AK025484.1, AL137479.1, AF218004.1, AK027103.1, AB048954.1, AL080126.1, AL133665.1, AF285836.1, BC001778.1,

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HFEBO17	48	852218	1 - 976	15 - 990	AW473576, A1089774, AW451782, AW295271, AA749033, BE440149, A1949946, A1469900, N68539, AW172942, A1872021, AW243195, BE858872, A1337875, A1376876, BE327191, BF939700, A1363093, A1471556, A1767259, BF114982, A1683261, AW028130, AW976177, BE349166, AA974484, AW027661, A1683115, AW614790, A1421188, A1281604, AW239182, A1750006, AW235809, A1493809, AA455090, BF878349, W37813, AV650898, F09284, AW193169,

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HFIJA29	49	839206	1 - 1261	15 - 1275	AW195543, AI051690, AI927925, AI051699, AI434786, AI675823, AW590850, W84675, AI971192, AA767204, AI767042, AW139875, AI521899, BF195790, AI250256, AA829382, N20059, AA215409, HI13567, Z38968, AA526451, H01273, HI13200, R08173, H01182, R82482, AW972928, AW207335, BF242637, AL031259.1, AL049844.7.
HFKFX64	50	566835	1 - 765	15 - 779	AI202664, AB051500.1.
HGBER72	51	826710	1 - 1302	15 - 1316	AI827764, AW963463, AV728410, AW964231, AV705122, AW956640, AW963895, AW956641, BF918640, AV702172, AI732151, AW958318, AW021917, AV759632, AW974932, AV702109, AV704541, AV704467, AV705086, AI962030, AV725237, AV711430, AW500029, AW956077, BF760919, AV762633, AV703573, BF804385, AW962006, AW970877, AW302909, AA905613, AV728369, AV763026, AV763058, AV702760, AI188390, AV729337, AA644090, AW969743, AI358384, AV729272, AV702343, BF750422, AW962942, AV726091, AW963497, AV703597, AW973992, AW960468, AV709273, AI305766, AV762454, AW966064, BF911056, BG236628, AW963542, BE063437, BF916934, BF347791, AW410354, AI017251, T05834, AV762982, AV711465, AW955841, AI279417, BE150580, AV762033, AA584489, AA904275, AL040054, AV757607, BE019467, AV758903, AV728425, AV703063, BF347740, AI963720, AW816516, BE294700, AL042373, BE395467, AW963489, BE178609, AA720732, AV712092, AW514662, AW069769, AW731867, AA574442, AV759557, BG029528, AV764259, AF246229, AC007731.14, AC005500.2, AC004033.3, AP001725.1, Z98941.1, AC005391.1, AL353653.19, U78027.1, AL034405.16, AC002301.1, AC004477.1, AL121897.32, AL133396.2, AC018638.5, Z83844.5, AC020916.7, AL035587.5, AL391241.21, AL096791.12, AL096865.28, AC002542.1, AL035422.12, AL117694.5, AC008543.7, AC005037.2, AC005841.3, AC005756.1, AL133551.13, AL109758.2, AL021155.1, AC006241.1, AC011464.5, AC008754.8, AL049643.12, AL138740.9, AC007536.9, AC020947.6, AC006211.1, AC079602.15, AC011495.6, AL136084.11, AL031685.18, AC004771.1, AL031432.1, AC008750.7, AC005701.1, AC008044.4, AC004840.3, AL139022.4, AL135927.14, AC007227.3, AC005821.1, AC004841.2, AC005399.19, AC007204.1, AB016897.1, AC083872.2, U02532.1, AE006462.1, AC011452.6, AL445685.17, AC006958.1, AC061709.25, AF196779.1, AJ009611.6, AP000692.1, AC005484.2, AC006345.4, AC005921.3, AL391262.3, AL512430.14, AC002563.1, AC004815.2, AC006055.1, AC006130.1, AC009756.9, AL139188.14, Z85987.13, LJ1910.1, Z81364.1, AL023807.6, AC009086.5, AC068799.14, AC004882.2, AK000254.1, AC013717.8, Z98257.1, AC008372.6, AL031295.1, AC000086.1, AC006543.7, AC008622.5, AL157369.7, AL136418.4, AL139054.1, AC008736.6, AC002425.1, AL157938.22, AC002565.1, AC005833.1, AC004824.3, AC010206.8, AC010422.7, AC002133.1, AL132653.22, AL109743.4, AP001693.1, AC008395.6, AL449209.2, U73024.1, AC011514.3, AL133545.10, AB055358.1,

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HGBGN34	52	648659	1 - 514	15 - 528	BF589439, AI127070, W95725, AI829385, W95768, AA732915, AI183361, AW967153, BE351006, BF941150, AI401364, AA321136, AI750875, AA321135, BG115775, AA878380, AA724102, AW962617, AA368761, AA455370, C00920, AC006208.3, AK024425.1, AB029496. 1.
HGLBG15	53	701990	1 - 764	15 - 778	AI377951, AA478899, AL521476, AI970420, BF571396, BF690972, AA478780, AI522149, BF571271, AA758425, AW956237, BF692249, AI954716, AW197154, BF576614, AI631753, AI829079, AI765476, H99846, AW274419, AI371713, AI951909, AW769338, BF516230, H19109, AI925973, AI932682, AI138219, AI301748, AI956119, AI755080, AI378559, AI080154, H05933, BF000829, AA031313, AW090099, AA736498, AA852791, AA852790, AA928061, AI699043, AI087918, AI468315, R56048, AI469137, AA248855, AW517996, BF541232, BF838384, Z38380, BF838382, AI760228, R55989, AI136597.1, AC005082. 3.
HHFEC39	54	609873	1 - 1288	15 - 1302	BE178297, BE891680, BE178296, BE178286, BE177969, AI817262, BE178117, BE220165, AW025400, BE178101, AI377829, C06099, BE178000, BF940116, AI422898, AI089906, AA583355, AA971743, AA044947, AA044943, AI076496, AI299481, AI376081, AA427892, H98616, AA618566, AA532381, AI080656, AA884934, AW341785, AA730790, AI139706, AW139974, AA992454, N99650, AI094082, AA483691, AA482694, D62553, AA001089, AI205651, R80152, Z36959, AI692586, AW958955, F10610, AA516076, AI311975, AI313288, AI379372, AI348747, D62481, AI610907, AA513037, AI611925, AI784325, BF054671, D62330, BF054866, BF057878,

HHSDI53	55	862028	1 - 1263	15 - 1277	AA149057, N71679, AA923038, AW969749, BF364372, R43511, D62106, D79281, N83844, T75442, BE168027, BE936283, F13013, BE043482, AI348789, D62153, BE168085, BE178420, BE177858, AL022726. 1. AW994394, AW151201, AW865905, AW865900, AW865898, AW866014, AW865891, AI755214, AW500684, AI754567, AI754105, AW576251, AL042373, AW613805, AW069227, AI923052, AI733856, AW341978, AA847499, BE062476, BE062478, AW576191, AW023111, AA420546, BG059972, AA449997, AW576490, BF911056, BF526964, BF828714, AV763026, AV763058, AW327624, AV732057, AA579179, AA410788, AI358712, AI634187, AU147162, BF691714, AW979087, AU146620, BE062545, AW516255, BF771349, AW328202, AW500029, BG250044, BE676019, AI792529, AW131356, AV703785, AW963663, AV763550, AI249688, AW958962, T74524, AW502873, AV695478, AW474168, AV762430, AI457313, AA828834, AI080307, AI962030, AV739518, AW275432, AW819125, AW026305, BG110162, AV730440, AI421950, AA513851, AI419337, AV730986, AW851405, AU144540, AW964231, AV741914, AV760508, AI038304, BE968744, AL135377, AI636734, AI361090, AV732950, AV754716, AV762009, BG036665, AI345654, AA578621, AW970896, AW021886, AA515048, AI569100, AA557911, AA501461, AL109936.10, AC078815.22, AL079335.29, Z69917.1, AP001760.1, AL136172.16, AC021752.5, AC009470.4, AC009269.6, AL049856.1, AP001169.1, AC008946.6, AC025262.27, AL356805.5, Y18000.1, AL162426.20, AC010271.6, AL121808.4, AL035587.5, AC005694.3, AC011462.4, AL355343.18, AL122001.32, AD000685.1, AC004020.1, AL158198.14, AC036103.8, AB050050.1, AC009570.13, AC011489.6, AC018636.4, AL391280.15, U63721.1, AC025166.7, AC004815.2, AL137128.4, AL109984.14, AC004797.1, AL133349.7, AC034242.5, AC005004.3, AL024498.12, AC010469.7, AC005529.7, AL138824.19, AC004805.1, AL136418.4, AL139054.1, AC017078.8, AC011737.10, AC083871.2, Z97632.1, AL359400.4, AC010654.8, AC005216.1, AC005052.2, AC018690.5, AL031670.6, AC007226.3, AC005932.1, AP000114.1, AP0000046.1, AL158207.15, AL121594.6, AC016995.4, AL034372.33, AF064861.1, AC008745.6, AP001717.1, AL136137.15, AF228703.1, AC068799.14, AC074121.16, AP000744.4, AC005086.2, AC007055.3, AL132653.22, AC011445.6, AC011450.4, AC004150.8, AC008760.6, AC012450.9, AF047825.1, AC006451.5, AC005103.3, AC004975.2, AL139316.5, AC005089.2, AL135927.14, AC007227.3, AL121972.17, AC034251.5, AC025593.5, AC011470.5, AP000356.1, AL034548.25, AC021876.5, AC008736.6, AC005725.1, AC010279.4, AC004647.1, AC087071.2, AC002115.1, AC006552.7, AF003626.1, AL137119.26, AC005338.1, AC020916.7, AL133240.3, AL161896.16, AL033378.12, AL359397.3, AF042090.1, AP001725.1, AL161727.15, AL391259.15, AC005200.1, AC009077.7, AC008962.8, AP001752.1, AC004867.5, AB053170.1, AL117381.32, AC010205.5, AC007722.9, AC008440.8, AL354707.17, AC074013.5, AC006449.19, AC007993.15, AC005029.1, AC005180.2, AC010605.4, AP002852.3, AP001727.1, AC004491.1, AL109614.28, AC009068.10, AC006348.3, AL096677.21, AC027319.5, AL445483.13, AC008649.6, AP000555.1, AF001549.1, AC011510.7, AC002316.1, AC006203.1, AC008403.6, AC004755.2, AL358815.12, AC006000.2, AC027644.9,
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					AC005098.2, AL159159.21, U78027.1, AC073838.6, AC002126.1, AC004771.1, AL121653.2, AC016587.7, AC005880.3, U91323.1, AL121601.13, AC005924.2, AP001053.1, AL022312.7, AC007388.3, AC004967.3, AL096840.25, AC004166.12, AL354932.26, AP001718.1, AC011465.4, AL353679.18, AC010463.6, AL121992.24, AC020754.4, AL049610.9, AC006441.13, AC006483.3, AC005620.1, AL136039.4, AL031295.1, AC008280.4, AC008397.7, AL161907.17, AL035422.12, AC004821.3, AC009220.10, AC004895.2, AC009509.7, AL137818.3, AC004125.1, AF031078.1, AC002352.1, AL356020.3, AC008474.7, AC002546.1, AL159997.14, AC007565.1, AL117382.28, AP001631.1, AC000082.4, AC007011.1, AC004813.2, AC008392.6, AF030876.1, AL137005.6, AC013719.8, AC005779.1, AC002401.1, AC002090.1, U91321.1, AC025280.4, AL445222.9, AC008569.6, AP003475.2, AL031228.1, U95090.1, AC016742.10, AL359711.18, AC068533.7, AL138836.15, AC004922.2, AC004840.3, AL021579.1, AP000694.1, AC005522.2, AF111167.2, AL022320.23, AL136220.14, AL031311.1, AL138741.13, AL354889.14, AC007664.12, AL356747.18, AC000003.1, AC007324.55, AL033397.7, AL359552.16, Z83844.5, AC021868.17, AL136170.12, AC016894.7, AL136228.8, AC007283.3, Z83308.1, AC005236.4, AP002342.3.
HISBA38	56	561711	1 - 1044	15 - 1058	AA465219, AW968392, AA463303, AL042753, AV759518, BF868994, BG029528, BF725761, AI963720, AL037683, AA129746, AL041924, AV682003, AV763460, AL138455, AL079734, AV757289, AI282253, AA669155, BF827410, BE252421, AL042853, AV760391, AA760655, AL079447, AV760389, AV762395, AA511105, AI049643, BG164617, AL137020.13, AC006581.16, AL122023.3, AL022322.1, AC007436.1, AC004706.1, AC068722.6, AB045357.1, AL035552.9, AP002532.1, AC022324.5, AL121869.19, AL109752.13, AC020559.4, AC008901.5, AC006017.2, AL354943.9, AC073897.6, AL353752.6, AL121823.12, AC005798.10, AF117829.1, AC018719.4, AL138832.10, AC010722.2, AL358612.8, AL163206.2, AL138879.10, AC090710.16, AC005250.1, AL365222.24, AL163213.2, AL133517.11, AP000493.1, AC005539.1, AL139150.12, AC003029.2, AC008766.4, AC009316.3, AC005002.2, AL133244.1, AL137818.3, AL031433.4, AC010081.4, Z97985.16, Z94722.1, AL590611.7, AC025253.20, AL137918.4, AL121652.2, AC073881.3, AC004998.2, AL355834.4, AL450345.6, AL163201.2, AL137840.12, AL049835.3, AL355143.17, AC006466.3, AC002457.1, AL132827.2, AC090957.1, AL049697.9, AL109939.13, AL133373.5, AC068723.5, Z98754.1, AL356278.8, AL355535.14, AC087315.21, AF212831.2, AL023876.2, AL359234.4, AL390205.17, AC007683.5, AC010140.3, AP002448.3, AC034148.9, AC027129.5, AL357272.10, AL023279.1, AC012372.4, AL022152.1, AC006287.1, AC006313.1, AC019155.4.
HJPBK28	57	638191	1 - 975	15 - 989	AL514305, AW293236, AW293727, H05931, AA641023, R38502, AI866873, AI401672, BF541631, AA809622, AA744709, AW300626, R60656, AW300632, AI559711, AW300658, BF591678, BF059502, AA433907, BF477999, AI143872, BF111052, AA923560, AI023411, AI580975, AW192719, AW080271, AI493147, AW242303, AW026654, AI569455, AA916462, AI281175, AI278657, AI419344, AI278903, AW305204, AI863945, BE856987, AA947586, AI954274, AI825314, BE551611, AU151920, AI422001, AI468214, BF061087, AI769184, AU159402, AI921752, AI984143, AW117197, AA446546, BE503412, AU153554, BE501613, AI656177,

HKABU43	58	838573	1 - 1905	15 - 1919	<p>AI075679, AI459572, AI739249, AU158932, AI962781, BE888552, AI340045, BE328347, BG120198, AW952038, AA933635, BF870463, BF828427, BE620781, AA304007, AA446399, AI137969, BC004415.1, BC007774.1, BC006291.1, AK023216.1, AK027624.1, AB040953.1, AB045381.2, AF216494. 1.</p> <p>BG035820, BG163860, BE779136, BG032640, BE546300, BG251357, AI890545, BF798002, AW957817, AW957894, BG164329, BE897914, AI064868, AW439699, BE868957, AI628884, AI538687, BG117638, BE075026, BE075028, BE877956, AI890859, AW241402, BF057808, AI962251, BE672376, BG254061, AI655998, W76094, AW593934, AW206368, AW070698, BF592891, BF855200, AI913939, AW242743, BE892303, W72889, AW510467, BE502137, AW852201, AW468485, AW242300, BE073158, BE073145, AI370901, AA076346, BE075023, BF761114, AI269861, BE927867, Z19251, BF229820, AA912859, AI962408, AA449269, BE869764, AA449405, AI125399, AI766912, BG230901, BF761369, R82858, BE927921, AI651447, AW852191, AW874171, AA313460, BE268347, AI440431, AW603030, AA322088, BE503487, AA373986, BE927954, BE677880, BE816387, BF679224, BE816422, BE927027, BF514420, BE297845, BE390905, BE535739, AV762904, AA564527, H29863, BE743929, AA369997, AA370398, AW957044, AA852197, AB018262.1, BC003633. 1.</p>
HLYGE16	59	651339	1 - 738	15 - 752	<p>AW469203, BF820842, BE218294, AW196671, BF447223, AI696980, AW236972, AI027666, BF798334, BF807954, BE217850, T59291, AI267964, R44968, AW445500, AW295686, AW291949, AI928514, AI823933, AU153630, AI139764, BE005097, AI948643, BF108749, AI126466, AW242784, R49472, AU160792, AW273139, AI273589, AW589378, AI274894, BF448101, AW510475, AI302181, AI400517, BE550344, AI365030, HI8516, AI206723, F09161, F09171, AI696176, AI992327, F09169, AW051573, AA847131, R86756, AU148421, AU154040, AI421825, AI696176, AI910733, AA868280, AA722823, BE504675, AI739531, AU152829, AA868452, AI911876, BE552250, AI168680, AI954643, AI913116, AW237207, BE502531, AI142459, D80575, AW002567, BF939794, D80957, AA702863, BF591908, AA455456, AI015316, AA922953, AV645338, AA514480, AI420243, R86981, AI420270, T59250, BF508779, R60233, R51570, AI580357, W86599, AA417873, AA085431, AA227559, AA852691, H57046, BF845379, AA192359, AI580716, AA455455, AA776815, AI140464, AA075296, BE300079, R27183, AA814809, AW105331, AA024748, D81100, BE620885, BF845381, R27182, BF912382, BE904044, BE904041, BE881261, BE965135, AI631590, BE551572, AI656791, AA282050, BG055430, AA937231, AC025594.5, BC009221.1, AK022910. 1.</p>
HLYGY91	60	658703	1 - 626	15 - 640	<p>AW294783, BE502344, BE222441, AI082255, AI031661, AI701563, BF431032, AW340159, AI250886, AA164268, AA113365, AW195764, AA813476, AI382168, AW044458, AI802164, AI149406, BF196258, AU155794, AA479123, AI167291, AI436306, AI224847, AI417116, AI709346, AI669258, AW772002, AA844518, AI282711, AI279738, AW195230, AW959069, BF002627, AI560087, AI286319, AI474555, AI092394, AA479124, AA243709, AI468637, AW991244, AA508073, AA243826, AI468739, T62160, AW975954, T61934, BE707630, BE169617,</p>

HMCFFH60	61	654853	1 - 429	15 - 443	<p>BF747189, BE832694, AA746981, AA328991, AK023448. 1.</p> <p>BG029413, AW410249, AL120205, AL527305, AW410004, AW411240, BE207947, AI348361, BG254821, AV717836, AI282565, AW015954, AI860745, BF970512, AI279557, BG250088, AI301063, AI887607, AW675703, AI277972, AI751711, AI610303, AW168266, AI954092, AW732241, AI199700, AI310726, AA533655, AI219656, BE047165, AI828679, AI829142, AI874208, AI741030, AI445423, AW339140, AW872712, AW872550, AI310725, BE675720, AW276596, BE049270, AA526998, AI300518, AI805844, AI814591, AA832328, AI927014, AA461097, AW337251, BE393698, AI453250, AW009901, AA522451, AI970703, AI147456, AI799656, AI866733, AI092937, AA526185, AI721118, AI017038, AI613235, AI339100, AW148657, AI538694, AW008035, AW269978, AI818220, AW130721, AI369774, AW778916, AA508660, AI285115, AW118526, AA280728, AI803837, AI078009, AI249388, AW274402, AA449775, AI741564, AI983830, AI953077, AI283484, W94943, AI186921, AA573897, AI078388, AA670351, AI423558, AW273429, AA745775, BE727124, AI591031, BF732731, AA903469, H98073, BF973696, AA628743, AW270071, AA987523, N91829, AI144428, AI081865, BG057107, BE797291, BE798645, AW899935, N34882, BG057959, AA065282, BE910046, W68425, AA132945, H26397, AA130713, AI535963, AA526103, BF436402, BF924840, AW189969, AA813305, F25776, R59097, BF793976, AW071554, AA102712, AL527475, W90665, AI533663, AL523124, AI246999, AW194200, AA677814, AW150820, AA004278, AL533350, AI983597, H25535, AI282522, BF941561, BE617576, BE613255, BF689583, AI521962, BF568937, R56834, AL524248, AI299507, AA805472, H46569, AW004802, BF828652, AA580297, AI364662, AA703237, AL521083, R56835, AA628330, BF570271, BE877417, AI365012, H56058, AA229754, AA229480, AA302484, AI918967, AI553849, AA373811, BF688841, AA853526, AI560300, AW470964, AA682774, BE858486, BF914567, AA496495, AI950742, R56673, AA526614, AA496620, R96820, AI972733, AA868647, AA644220, AA447147, AA228723, BG015338, AA887190, AA229233, AI690364, T51235, AW075387, AA953331, BG112305, AA713800, AI094450, H61569, BF375945, AA449063, AI026692, AA159983, BF914242, T73441, AI720505, BF336367, F31462, AI827198, AW772776, AA614196, AI300639, F18178, R10734, BF336341, BE548560, AA858412, BF912964, AA229962, AA872093, AI015741, AI051521, AI203695, AA978132, AA988865, AA365973, BF948395, AI690503, W68523, AA229458, Z38471, AW262508, AA736839, BF690492, BE181244, AA007293, BE122672, AW182880, BF947488, AW771037, AI350873, AA557419, BG016041, AW515865, AV697048, R56672, T24559, BF183570, AA923506, AF189289.1, BC000702.1, AF176006.3, AF192559.3, AF151822.1, AK022783.1, AF090943.1, AL512733.1, BC005402.1, BC006091.1, AL359583.1, AK027129.1, AK025414.1, BC007462.1, BC001967.1, AB060832.1, BC008842.1, AK025435.1, AK025113.1, M85165.1, AK027103.1, AL353940.1, BC004951.1, AL050172.1, AL389947.1, AF056191.1, AK027082.1, BC003651.1, AK027136.1, AL389935.1, BC009398.1, AL137530.1, BC002370.1, Z37987.1, AK000653.1, AK027102.1, AF245044.1, AL080146.1, BC008836.1, BC004290.1, AL137459.1, AB056372.1,</p>
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HMI/AK10	62	562774	1 - 1050	15 - 1064
HMIBD93	63	634227	1 - 1309	15 - 1323
				<p>AL045780, AW590225, AW970178, AI376010, AI401550, BF940066, AW072377, AI362753, AW005335, AA524093, AW575918, AW592039, AI097125, AA514373, AI424874, AA490412, R55849, R89670, AA502607, AI361469, W60204, R45456, AA906578, BF940638, H17344, W35351, AA757050, D52329, AA309188, AA490314, AA479853, R45361, AI929199, AA456776, AA394201, AA806240, R55770, W32915, AI380329, Z38945, AI003744, AA411018, R52109, AI471415, AA863370, AW953330, AW866322, BF921092, AL048656, BF038804, AW238730, BF904194,</p>

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					AF090943.1, AF218004.1, AB055374.1, AK027213.1, U91329.1, AL389935.1, AB049892.1, BC001418.2, AF026816.2, AL122110.1, AL359941.1, AL137283.1, AK025391.1, AL136640.1, AF217966.1, BC008284.1, AB060908.1, AF217987.1, AK025407.1, AB060916.1, AK000212.1, AK026855.1, AL137529.1, BC001098.1, AL136747.1, BC004556.1, AK026551.1, AY026527.1, AL117435.1, AL136787.1, AL136882.1, AL136805.1, BC006195.1, X98834.1, AK026408.1, AB055361.1, AK024594.1, AL136692.1, BC004529.1, AL050024.1, AL049430.1, AL133640.1, BC008280.1, AF177228.1, AF183393.1, BC000090.1, AB052191.1, AL080127.1, AF090886.1, AF056191.1, AL050138.1, AL137479.1, AB060912.1, AL512684.1, BC006164.1, AK027200.1, BC000317.1, AK000323.1, AL136864.1, AL080126.1, BC003122.1, AK026624.1, X72889.1, BC001470.1, AB048953.1, AF111112.1, AL050393.1, BC003052.1, AL137556.1, BC002839.1, AK026583.1, AL136786.1, BC002519.1, AK026630.1, AK026526.1, BC007198.1, BC004191.1, AK025383.1, AK026592.1, BC008365.1, AC044797.5, AL389939.1, AK027116.1, AK024524.1, BC002457.1, BC003627.1, AB048994.1, AK026353.1, AL122050.1, AB055370.1, AL512754.1, AK025209.1, AL389957.1, BC008485.1, AB056809.1, AL080162.1, AF090903.1, S77771.1, AJ012755.1, AL136749.1, BC007021.1, BC009341.1, AL137463.1, AK026542.1, AF090934.1, AL137526.1, AL136767.1, AL049382.1, AL117583.1, AL512733.1, BC004195.1, AB056420.1, AK027204.1, AK025431.1, AL117585.1, AK027160.1, AK000291.1, AL359601.1, BC007998.1, BC007534.1, AF352728.1, AL080148.1, BC001206.1, AL133565.1, AL133112.1, AK000486.1, AK026532.1, BC004923.1, BC008387.1, BC002539.1, AL110196.1, BC008899.1, AK026642.1, BC004265.1, AB060837.1, BC003110.1, AL133081.1, AK026959.1, AL136915.1, BC003548.1, BC008417.1, AL353935.1, BC006412.1, AK026506.1, X53587.1, S78214.1, AK026608.1, S61953.1, AF078844.1, AF100781.1, AF358829.1, AF097996.1, AL133093.1, BC006509.1, AK025967.1, AK026480.1, AB063070.1, AK024545.1, BC002643.1, AF218014.1, AL390154.1, D89079. 1.
HMWJF53	64	758158	1 - 2274	15 - 2288	AL520010, AL521909, AL521910, AL520011, AL524545, BE300370, AV705059, BG248217, BE250720, BG031597, BE250726, BF315262, AA524095, AL047607, BE871170, BE871652, AW402124, BG168243, BF343529, BF038946, BG168526, BE394575, BE560712, AL047608, AW574683, BE295900, AW575182, BE543224, BF205183, BF569039, AI498950, AA779842, BG115998, BE301593, AI453393, BE018107, AI628521, AI884947, AW474397, AI814310, AW166122, AA566001, AW026116, AI948522, AA614479, BE018086, BE540280, BF663008, AA886908, AA777831, BG115717, BF114669, BE393994, AW182896, AI707682, AW770121, AI673011, AI206429, AI092365, AI460223, BF793598, BE672939, AA522788, BE294120, AI061456, AI223983, BE349928, BE787943, AI628305, AW674834, AW673969, BE300207, BE328440, AW628266, AW008044, AI002213, BE677129, N93597, AI634627, AW402526, AI285191, AI283397, BE301576, AI609714, BF876020, AI884999, AA970842, BE546816, W25474, AW149381, BF111468, BF569107, AW057693, BE512675, AW083509, AI810870, AA079217, F31081, AW166408, AI436188, BE676788, AA766757, BF238323, AW275173, AI632859, AI903523, AI634638, C03499, AL524544, F37056, AW403327, BF211595, AA995404, AV749582,

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HNECL22	65	799541	1 - 2696	15 - 2710	<p> AI114773, AV708528, AI174910, AI114822, AI065060, AI305115, AI207643, AV701391, AI114681, AV709313, BE221818, BE677761, AW953876, AI743950, AW953822, AI589902, AA886890, BG236178, BF591137, AW516553, AI027819, BE550036, BE675476, BF684928, AI193143, BF447812, BE551855, AW003234, BF115226, AI871594, BF589312, AW968116, AF074701, BF115270, BG149200, BE669558, AI636074, AI186791, AA453707, BE348795, AA459528, AI223053, AW293894, AI765135, AA465507, BF000591, AA837316, AI342451, AW043650, AI344323, AI015206, AA906314, AI675107, AI374588, AW629315, AI367638, AI285946, BE348663, AI126552, AA459297, AI082415, BG058812, AA827913, AI186011, AI493737, AA579658, AI439599, AI064752, AI580179, AI191379, AA057245, AW978758, AW197214, </p>

					AI273440, AI719270, AW084532, AW167688, AW614430, AI400741, AI128538, AI261492, AI936977, AI694244, AI392728, H56267, BF116001, AI215180, AI091065, AA478846, AI141102, AI439574, AI262021, AI439573, AI376357, AI347510, AA477065, AI278176, AI094135, AI076690, H04614, AI439592, AI065009, BE675923, N70205, AW408556, AA447775, AA228012, AI762994, AW953823, N90124, N91093, AW016927, AI914209, AA116051, AI092840, AW661858, N69573, AI246680, AI694653, AI863991, AA779767, N72602, AI912117, AA313800, AA962551, H93303, H51282, BF929847, AI346284, AI521889, R63715, AA342837, AA479942, BF732747, AA465396, R74142, AI252135, AA454473, R92925, N77670, H04592, AI141789, AI816723, AI204646, R63761, W78774, N58304, AI655820, BF062215, W02412, BF512624, BF855761, H78806, AI341922, H73792, BF591907, AA481390, T50082, BF834392, BF742347, H73779, R74118, AI630220, AA811993, N39344, R96556, AA939056, H56071, BF989488, N63741, H51859, AA927199, W26847, H72984, R91506, T50066, H66255, AA908836, AI830053, AI351268, H49848, AI858083, H92164, BF814618, AA333775, AW303217, AI864385, AI382793, AW301915, H27595, H88107, AI114652, AA813528, H61504, AW952524, Z20785, R91276, R67005, H73791, AI088971, R65623, T50019, R68277, AI925217, AA683337, AW385241, H88106, AI858078, N52417, AI612859, AI201887, H40449, N94063, H66660, H71977, AW196541, H69793, H66661, AI133036, AI381768, AA369715, R74028, BF446051, AW005811, AW151526, AI167780, AI217406, R96511, AA627870, R74049, H92165, H91341, AI073965, AI133696, AI127197, H91424, H27525, AA419172, AI310713, AA410653, R68314, AI127450, T50002, W04455, H62478, AA031272, N76050, AA916572, AA370598, BE856933, AY032628.1, AF155660.1, AF223466. 1.
HNFAC50	66	815676	1 - 1428	15 - 1442	BF888349, AW071725, AA743534, BE783671, N57590, N57604, AW305107, AV750698, BG003734, D45491, AA485566, BF358205, AA504633, AW026010, AW025529, BE512979, AI885090, AI475932, BF108880, AW050607, AA886335, BF368455, BF109416, AA662803, AI375435, AI832600, BE906368, AI393408, AI056120, AI244837, AI056614, AW449834, AA657904, AA922036, AA643750, AA878578, AW197722, AW058170, AW748250, AI560410, AA485405, AI749095, BF436871, AI720931, AI446208, AF308287.1, AL137403. 1.
HNGEA34	67	815678	1 - 1089	15 - 1103	AP000531.1, AC003064.2, AC002471.5, AC005374.5, AL512624.4, AL391119.8, AL132657.33, Z83839.1, AC008443.8, AL049849.1, AP001229.3, AP001214.3, D87003.1, AL133173.19, AC026273.7, AC018696.4, AP000547.1, AL078472.3, AC002041.1, AF254983.2, AL163201.2, AC013734. 4.
HNGIV64	68	561572	1 - 1033	15 - 1047	AA595803, AV653403, AI886084, AV684943, AV695480, AI363970, BF848469, AW380640, AV651029, AL049541.24, AC009475.4, AC020910.5, AC008556.5, AC067941.7, AC004967. 3.
HNGKT41	69	836061	1 - 1034	15 - 1048	AW862214, AW859811, AW862215.
HNGNO53	70	836063	1 - 811	15 - 825	R37935.
HNHCT47	71	634691	1 - 607	15 - 621	AA469441, AA328289, AW972888, AW968156, AA502813, T09124, AI821722, AI732162, AI821172, AI792063, BE146613, BE146622, AU119400, AU119141, AA736713, AA572983, AL120694, BE392609, BF676104, AA598663, AI798242, BF346320, AA525409, AA834799,

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HNHKL174	72	777856	1 - 803	15 - 817	<p>AC058791.3, AP000352.2, AF011920.1, AC004703.1, AC018711.4, AC008623.4, AC006946.20, AL035689.26, AC007383.4, AL400877.1, AC004953.1, AL035420.15, AC023880.5, AL159140.4, AD001527.1, AC002394.1, AL035072. 16.</p> <p>AV728309, AV707794, AV706891, AV725423, AV704740, AV709273, AV702172, AV701844, AV734690, AV683012, AV705525, AV734974, AV703460, AV702625, AV702760, AV705299, AV732961, AV726754, AV702857, AV706410, AV728425, AV764444, AV704467, AV762687, AV759295, AV725431, AV707135, AV762870, AV727065, AV702659, AV759719, AV759507, AV762973, AV761810, AV706448, AV760057, AV761107, AV729881, AV759717, AV761489, AV761613, AV729947, AV707230, AV762505, AV722143, AV755512, AV763558, AV763410, AV704541, AV761412, AV702343, AV706814, AV709625, AV701499, AV762015, AV760106, AV703137, AV707043, AV762706, AV762631, AV707067, AV763298, AV762242, AV706025, AV733894, AV762852, AV764465, AV756702, AV709025, AV728138, AV701739, AV757334, AV705436, AW963592, AW955724, AV703844, AV704497, AV707654, AW962651, AV729090, AV702738, AW962006, AV755888, AV760048, AW957644, AW962942, AW963486, AV762009, AV761661, AV709897, AV729272, AV708388, AV712770, AV702109, AV711430, AV762082, AV705086, AV763847, AW958318, AV760908, AV725558, AV764034, AW963489, AW961593, AW966064, AW963552, AV701613, AW960468, AW956077, AW963463, AW962444, AV762067, AW953953, AW962268, AV727583, AV756508, AV703218, AV759768, AW961330, AV757526, AW963625, AV729411, AV705020, AV688823, AV710733, AV760724, AV683668, AV759352, AV763444, AV705532, AC002302.1, AL031273.1, AC005067.2, AC003982.1, AL022165.1, AC005225.2, AP001711.1, AC002470.17, AC012476.8, AC009060.7, AF196969.1, AC007917.15, AL354932.26, AC004148.1, AL359091.10, AC006059.3, AC005207.1, AC006312.8, AP001689.1, AC004824.3, AC007000.2, AC004840.3, AC011497.6, AC005229.1, AC006077.1, AC006480.3, AC005058.1, AC022543.2, AC004263.1, Z82184.1, AL445483.13, AC005632.2, AF134471.1, AC005859.1, AC002477.1, AF258545.2, AL024498.12, AC010458.5, AL136418.4, AL139054.1, AC002425.1, AC003007.1, AC006111.3, AC003692.1, AL096763.14, AL357497.17, AP001714.1, AL109743.4, AL118497.9, UI5177.1, AL391384.18, AC005282.4, AC004089.25, AC011455.6, AP000089.1, AL132640.4, AP001630.1, AP000300.1, AC010319.7, AL136131.15, AC009137.6, AC010679.6, AC005015.2, AC018644.6, AL049761.11, D87675.1, AC007308.13, AL161779.32, AC005726.1, AC008599.6, AC009470.4, AC006121.1, AC011479.6, AP000113.1, AP0000045.1, AF222686.1, AC004659.1, AC004000.1, AL158040.13, AC008745.6, AC004878.2, AL121914.31, AC004166.12, AC018755.3, AC019014.1, AC007003.4, UI7576.1, AC010326.6, AC005052.2, AL354707.17, AC006038.2, AL008718.23, AL022476.2, AP001725.1, AL031282.1, AC007938.1, AL133448.4, AC018663.3, AC007216.2, AL117258.4, AC004867.5, AC006014.2, AC008891.7, AC007684.3, AC011489.6, AL353716.18, AL022302.10, AF312915.1, AC020906.6, AL354696.11, AC007991.7, AC007151.2, AC013726.7, AL359828.13, AL157838.24, AC005089.2, AC020928.6, Z68273.1, AC007021.3, AL161893.24, U91323.1, AC004149.1, AC008068.4, AP001717.1,</p>
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HORBS82	73	638293	1 - 1111	15 - 1125	<p>AA716165, AW014086, AI675797, AI915560, AI093476, AI619556, AA346257, BG170965, AI674463, AI656676, AW962578, H26720, F32296, F33070, F24055, F23333, F32966, AI370391, AI446003, AI560806, AA857847, AW130863, AI282355, AI241901, AI889818, AI611743, AW243878, AI619502, AI630928, AI953765, AW083804, AA504514, AW081179, AA814782, BE967273, AI554821, AI620056, AI285735, AW262983, AI355849, AI245332, AI018686, AI635464, AW183620, AW020095, AL036187, BE966547, BG105445, AL036509, AI680498, BF970652, AW149869, AI433611, BG181012, BF914091, AI271796, BE621256, AV702932, BE962857, AI824557, AA555145, AA449768, AL513697, AW089009, AI368579, AW020592, AW130403, AL514823, AW022494, AW020288, BE785348, AL513817, BG108350, AW073996, AA937558, AV724929, AI863321, AI684127, AW075648, AW082997, AL514035, AI811911, AW168485, AV705811, AW083825, AI972170, AI537045, BE964576, AV734654, AI815239, BE964792, AI434833, AI301507, AI687168, AI289791, AL513693, AA808175, AL513911, BF763498, AI918634, BE965014, AI623736, BE965891, AI952249, AI862139, AW081343, AI923768, AL513809, AI050666, AI367210, AW170725, AA835947, AW025412, AW082040, AW025279, AW079045, AL513789, AI432532, AI874189, AI289608, AI536685, AI680113, BE965031, AW189268, AW827211, AW082623, BE965053, AI560023, AI439762, AW029611, AI978720, H89138, AI801592, BF339322, AA603709, AV659322, AI925404, AI627714, AW008090, BE735370, AW075519, AI866691, AI559737, AI921753, AI718161, AI582932, AI872545, AI446809, AA904121, AI251434, AI784214, AL514357, AW193467, BF338782, AA983883, AA480074, AW075482, AL514473, BG179586, AL514557, AI804983, AI697045, AL514691, AI924971,</p>

				BF338027, BE299813, AW085373, AA514684, AL513553, BE966571, BE963909, BE964150, AI915207, BG178911, AI469516, AW151835, BF968679, AI475394, AI568114, AL514627, AW151850, AI457369, AI824648, BF038804, BE965481, AI375730, AW085667, AW192245, AA493923, AW059713, BF025686, BE878953, AI640379, AI498579, AI888665, AL038778, AW162189, AL515235, AI744243, AI691088, AW073868, BE964967, AI933940, BE613727, BE875442, AL514929, AW161202, AI961590, AI680435, BF968622, AI873638, AI863082, AL514455, AI915295, AL514101, AI859644, AW088944, AW089327, AI658566, AW806761, AI564186, BG179099, BF526494, AL513723, AL514983, AI811168, AL514089, AL514871, AW082060, AI925463, AL513779, AI952542, AI285439, AL514409, AI812107, BF971669, AI619751, BF904248, AI635478, AL514721, AL513999, BG105501, AI628325, AW020710, BF525392, AL513823, AI745713, AL034419.22, AK027081.1, BC002777.1, BC004926.1, AB063046.1, AF090900.1, AK000598.1, BC003591.1, BC003682.1, AK025099.1, AB047953.1, AL353957.1, AC004987.2, BC002495.1, AK025906.1, AF303581.1, AF178432.1, AC008592.4, BC003410.1, AK026528.1, AL133088.1, BC008708.1, AB060826.1, AB048910.1, BC004943.1, AB063071.1, AL050170.1, BC000799.1, BC005805.1, BC006164.1, AL136789.1, AL162062.1, AK026045.1, BC007517.1, BC003687.1, BC009311.1, AB052191.1, AL121916.14, AL389935.1, BC001294.1, AK027121.1, AB050510.1, AK026590.1, AL080074.1, BC001470.1, BC001236.1, BC002373.1, BC001328.1, AB060929.1, AL353956.1, AK000636.1, AL359624.1, BC004908.1, AB055303.1, BC007522.1, AB060887.1, BC006832.1, AJ004832.1, BC000066.1, S77771.1, AJ406937.1, BC001418.2, AK025375.1, AF179633.1, BC008649.1, AF245044.1, AK000432.1, AF141289.1, AB049892.1, BC008718.1, AK024974.1, AK027142.1, BC008983.1, AK007383.4, AL133640.1, AL137523.1, BC007453.1, AL137480.1, AP001346.1, AL110221.1, BC000054.1, AL137495.1, AL136925.1, AC004213.1, AL161802.15, AC006112.2, AL354828.12, AL035458.35, BC009033.1, BC001774.1, BC007248.1, BC005816.1, AL122111.1, AK027193.1, AF369701.1, AK026551.1, AL035407.15, AL356859.12, BC001801.1, BC002752.1, Y00093.1, BC006345.1, AK024747.1, AB049758.1, AK026855.1, AK024538.1, U77594.1, AB060229.1, BC007031.1, AK026542.1, AL080057.1, BC000008.1, BC000386.1, AK026480.1, AK025084.1, AL157464.1, AL080127.1, AL137705.1, AL121601.13, Z49258.1, BC002733.1, BC002491.1, AB049848.1, AF217973.1, AF239683.1, BC002409.1, AC006451.5, BC003105.1, AL157360.8, Y14040.1, AL389978.1, BC002839.1, BC009395.1, AF067420.1, AP001731.1, AK027154.1, AB060881.1, BC002471.1, AL137711.1, AB050431.1, AK024855.1, AF274348.1, AF274347.1, AF352728.1, BC006087.1, AL353940.1, AF004162.1, AL050092.1, AL136787.1, S76508.1, X53587.1, AF232935.1, AL132985.4, BC004481.1, BC008507.1, BC002344.1, AF094850.1, AP001666.1, BC001099.1, BC004431.1, AL133010.1, Z94277.1, AL137556.1, BC002399.1, BC003104.1, AK025209.1, AF110640.1, AF120268.1, BC008788.1, BC007534.1, AK026038.1, AC008507.8, AC026307.16, AC007172.6, AL031732.8, AC017082.4, AK026749.1, AF012536.1, BC007053.1, BC009026.1, BC002444.1, AF218014.1, BC001785.1, AC002540.1, AF225424.1, AK025414.1,

					<p>AF217987.1, BC007355.1, AK027164.1, BC008723.1, AL512689.1, AK000753.1, AL121952.18, AL133062.1, AL136979.16, U91329.1, AF095901.1, BC007034.1, BC002958.1, AL080060.1, BC007389.1, AK026395.1, AL356747.18, AL590076.3, AF022813.1, BC004925.1, AC008897.7, AL137461.1, AL080110.1, BC006196.1, AL117585.1, AL136843.1, S69510.1, AF044323.1, AK026526.1, BC002574.1, AL355143.17, AL157878.11, AC020956.6, AF358829.1, AF028823.2, AF162270.1, AB060893.1, AK026518.1, AF177336.1, AK026793.1, AK026630.1, BC006287.1, AL136586.1, AF321617.1, BC006465.1, AK000647.1, AK026649.1, AC005968.1, AK000450.1, M19658.1, D83989.1, AL049464.1, AF073483.1, AF217989.1, AF217966.1.</p>
HOUDE92	74	580866	1 - 1270	15 - 1284	<p>BE736091, BF237553, BE781264, BF686547, BE313480, BE872070, BF313936, AI138711, AI348027, BE502126, BE258631, AA524244, AW873570, AI982983, AI367855, AI052179, N90758, AA325647, AW419076, AW873111, AW008195, AI304671, AI367495, AW964887, AI609692, AA019213, AI279349, AI581275, AI224904, AI141287, H14110, H41440, AI017367, H29060, H29163, AA482386, AI471043, AI742262, AI262559, H52568, AA872715, R60248, H06091, AI041676, BE856821, H86160, H86771, AI241156, AA872384, R60761, AW131262, T31006, H56455, H95225, AA535480, AA678522, AA953998, R93546, R47352, BF968234, C04826, N39943, AA779062, T31180, H69216, AA017105, AA738315, AA019233, C04344, C05015, H17526, R99865, H84704, AW025505, AA057567, N72695, H86419, W02476, N27200, AA001522, AW194286, AI264419, AI220672, AI290418, T30927, AI620442, AA985424, R49316, H86772, AA725465, R91429, R93547, AA017106, AI074855, H95701, H69217, H95226, AW188581, AI678424, AA057566, AA326095, AA976949, H56456, W57713, AW166317, Z42112, AA775239, AI8664069, AA918031, H85105, AA015626, AA977988, AA429622, R99866, H14085, AI000910, AI431360, Z38375, W57838, AA015625, R57558, AI949351, AI262422, AC005865.1, AF217967.1, AK027366.1, AC005912.1.</p>
HOUFS04	75	771564	1 - 2913	15 - 2927	<p>AL537447, AL537448, AU120015, AU131841, AU141085, AU134821, AW612291, AU124089, AU139563, AV727622, AV727623, AU133175, BE788006, BF185243, BG257827, AU136898, BF184853, AI627486, AV705533, BF590192, BF577029, AL118692, BG257588, AU153504, BE326681, BF028316, AU154556, AL135492, BF431327, AW369693, BF216161, AI740778, AW239374, AW150161, AU150952, BE972535, AI274815, BG011960, BF210890, AV749930, AI091535, BF241838, AA88926, AL121256, BF104153, AI795954, AW513116, BF185887, AI433845, AI343701, AU158077, AI277331, AW268991, AA680329, AU155657, W28248, BF594656, BG000296, AI457635, AW661960, AI263881, W28288, AI434627, AA010836, BG012652, AW129101, AV748375, AW302270, N52145, BF219378, AL518583, AW297250, N75868, AA857253, AL120585, AW994991, AA011013, W26416, N22080, AI015118, H02915, H39966, AI039206, AA449595, AV684996, AL518582, AA953973, AI440447, AA016111, H03822, BF825307, R84685, BE702037, R84707, AA019431, BF825558, AI275797, BF826197, AA330443, R76642, R76568, BF844855, BG011970, AL120540, D79415, R85665, R84949, AW296468, AI539211, H95551, H28472, R33103, AI696200, BF210071, T54671, AA604416, R33200,</p>

				AA011115, BE177754, BE177752, R85648, AA058782, BE177756, BF058858, R31662, BF825522, AA016056, BF054961, AW628524, BE218727, T54758, AW609383, AW609416, AA020805, AA419376, D62124, BE541542, AA058678, AA021571, A1610553, X85615, AA913581, BF825480, BF793324, X85616, BF970449, BF814447, BG180996, BE548914, BE536058, BF038131, BG256592, BE880182, BF032910, BF822127, BE874133, BG029053, BG257807, BG163618, AA715307, AW827276, AL036361, BG118430, BE047852, BF338723, BE876033, BE889314, BE875243, A1345543, AW673679, BE877846, AW162194, BF338002, BF968017, AW163464, BE875028, BF339322, BE877769, A1364788, BF811805, BE612681, BF339310, BG249582, BG032036, AW772685, BF032768, BG254745, BF911528, BE622183, BF814420, BE785868, BG180034, BF811793, BE910373, BE881315, AA809974, BE047859, BF680133, BF812936, BF340323, BE904178, BF885082, AW020693, AW020710, AW827289, BF344031, AL514691, BF982040, BE047952, BG120816, BF343521, AV727226, BE874997, BF970768, AW151979, BF885080, BF344733, BF812933, BG164371, BE047691, AL121286, BF856052, AL039086, BF244608, A1371228, A1623941, BG106619, A1500061, BE889355, BG122101, BF339594, BG251104, BE048179, BG113662, BE894455, BE881131, BG165879, A1371251, BF310166, A1923989, A1581033, BG036846, BF727212, AL045500, A1567971, BG169383, BE887488, A1343091, BF812937, BF751997, AB011182.1, AK002207.1, AK001949.1, AL139377.8, AL117457.1, AK025391.1, AB056421.1, AL122110.1, AK000083.1, AL122049.1, AF218031.1, AL162062.1, AB052200.1, AF111112.1, AF143723.1, AK025015.1, AL162083.1, AF217987.1, BC008719.1, AL389935.1, AL133606.1, AL512719.1, AK000618.1, AL359596.1, AK025254.1, AL162006.1, AL133557.1, AL110225.1, BC003687.1, AK026642.1, AK000212.1, AL136915.1, BC006525.1, AL110196.1, AL080124.1, AL137526.1, AL512733.1, AB060916.1, AK026927.1, AL512765.1, BC004556.1, AK024538.1, AL050277.1, AB060873.1, BC003682.1, U80742.1, AL133072.1, AK026593.1, AL050024.1, AK027142.1, AK027096.1, AL122050.1, BC008899.1, AK026959.1, AK026434.1, AL050393.1, X65873.1, AL137429.1, AL357195.1, AK025857.1, AB056420.1, BC001963.1, AB060852.1, AL137533.1, AK027116.1, AK024594.1, AK026647.1, AF260566.1, AK026592.1, AB052191.1, AK025119.1, AL136843.1, BC002342.1, AF090901.1, AL117435.1, AB055361.1, AF078844.1, BC001418.2, AK026480.1, AL133080.1, AF151109.1, AF217982.1, AK025708.1, AL133077.1, AL389939.1, AK026629.1, AL122121.1, X82434.1, AL049283.1, AL049382.1, BC005168.1, AF183393.1, AF090900.1, AL137550.1, AL442082.1, AB062978.1, AL359618.1, AL137476.1, AK027164.1, BC008488.1, AL162008.1, AK025967.1, AL137478.1, AB048954.1, AB048974.1, AL049452.1, BC005890.1, AK025798.1, AB063071.1, AL110221.1, AL136789.1, AK000391.1, AF146568.1, AF207829.1, AL133104.1, AL049996.1, AK026086.1, AB062942.1, AF218014.1, AB047623.1, AL353957.1, AK025414.1, AL512746.1, AB047801.1, AB056809.1, AL136893.1, AF125948.1, AL359620.1, BC003122.1, AL137292.1, BC006195.1, AF227198.1, BC004958.1, BC007326.1, AB060912.1, AF090934.1, AF090943.1, AL137488.1, AK026744.1, AB060908.1, AK026741.1, AB055352.1, AF218034.1, AL050146.1,

HOUHI25	76	888279	I - 1235	15 - 1249	<p>AL353956.1, AL136805.1, AK026600.1, BC008365.1, AF271350.1, AL162002.1, AL117648.1, AL136884.1, AB063070.1, AK000445.1, AL049430.1, AK026045.1, AK026613.1, AB063088.1, BC006180.1, AK027146.1, AL122098.1, AL512689.1, M92439.1, AL110280.1, AB062750.1, AK026542.1, AK000718.1, AF348209.1, AL049464.1, AL049339.1, AF217966.1, AL137271.1, AL137529.1, AL096744.1, AK000323.1, AF111847.1, U91329.1, S78214.1, AF026816.2, BC008382.1, AF091084.1, AL136692.1, AK026057.1, AL133640.1, AB060879.1, AK025084.1, AK026649.1, AB060825.1, AL137574.1, BC003548.1, AL050108.1, AB047904.1, U78525.1, AY033593.1, AB048953.1, AF104032.1, AL096751.1, Y16645.1, AL080074.1, AL512684.1, AK026583.1, AL137560.1, BC001967.1, AB056427.1, AL049466.1, AF202636.1, AK025092.1, AK027213.1, AK025312.1, S7777.1, AF056191.1, AL136787.1, AK000486.1, AK000247.1, AF230496.1, AL133560.1, AL137463.1, BC000094.1, AL359583.1, AB055315.1, AK025209.1, AL137538.1, AL133075.1, AL050149.1, AL136892.1, AK000690.1, AL050092.1, AK026624.1, AK026506.1, AL137521.1, AF061573.2, AK026462.1, AK026532.1, BC004926.1, S61953.1, AK026408.1, AK000614.1, AL512750.1, AK027114.1, AK024588.1, AB060826.1, AL049938.1, AK026528.1, AL117587.1, AB050510.1, AB048919.1, AK026626.1, AL133081.1, BC004951.1, AL050172. 1.</p>
					<p>AW274757, BE979499, BE875104, AV728303, BF671975, AV727326, AW631495, BF983857, BF248008, BF115795, BF212520, AV717938, BF571136, AV728044, AA976644, BF679046, BF477635, AI907366, BF570689, AA903720, AW972361, AA524665, BE220972, AI984786, BF979139, AI692731, BF213370, AI907373, BE439966, AI268254, AI907368, H08416, BF700421, BF541919, AV705494, AW978976, AI332994, BF540780, BF247753, AI907374, AA826200, AA701660, AI656122, AI657191, BE763093, AA936326, BF572170, BF131794, BF195177, N79377, AV715209, AI950823, BF093269, N88408, BF439990, BE159589, AA772723, N86348, AA399432, BF575806, BF984268, AW206085, AI268253, AI421859, BF680498, AI916469, BF344634, AI420669, BF670000, BC008478.1, AF126020.1, AC004839. 1.</p>
HPCAL26	77	762822	I - 3083	15 - 3097	<p>BG164171, BG171313, AW338908, BE327883, BF058325, BE856282, AL525344, BG027433, AA621714, AL047905, AW780148, AI633775, BE973735, AW438611, AI755212, BF381979, AW337238, AI337968, AI963595, AW572336, AA432021, AW471145, AW069566, AA448477, AI936887, BF108841, AI829408, BE748629, AI042324, AI955816, AI421409, AA758227, AA814190, N91448, BF030663, AW192439, AI683517, AA417975, AI129364, AI561083, AA418135, AI088536, AW628520, BE327874, W72280, BE878275, AI348236, AI088467, N26180, AI628017, AI066421, AI346288, AI142951, AI308778, AI446338, AA458899, AI078536, AI346382, AI753070, AA923036, AI431409, AI087120, AI341640, AI567761, AI371263, AI754690, AI285250, BE619821, AA630948, AI354829, BE620033, AW294799, W45027, AI902379, AI537262, AI866883, N27411, BE876666, BF338393, AI963351, AI081820, BE395375, BE620777, BE906942, AI811301, AI627940, AL538418, AA602460, BE548617, BF038519, AW205903, BF893012, W76307, AA701166, AL525384, AW023777, AW956752, BE122762, AA128212, AI094190, BG163634,</p>

					<p>AI371264, AW856743, AW380612, R81003, BE905099, N23359, BE619502, AI589929, BE905025, AW469305, AI254707, N67744, BE619375, AA961234, BE906749, BE784570, BE909607, AW387716, BE904894, BF695306, W25857, AA134565, AW044064, AW802015, BE673511, R76732, AI392841, BF509258, BE503655, BE880509, AI299995, AA304323, AI074674, BE788330, AI086914, AA383490, AW630488, AA977524, AW802237, AA296648, AA431796, AA047280, AA813746, AW236745, AA193605, AA127172, AW080334, AI696769, BF437027, BG167009, AW361256, BE938690, R76394, BE218539, R39674, BF475923, BE843065, AW819110, AW819102, R39598, R80801, AI540488, AA134564, AA193568, AW818988, AI245052, N55215, BG120706, AA307251, BG119547, BE843137, BE816369, AW957969, AA776346, AW819052, C01782, AW517411, AI906011, AA303350, T24798, BF799939, AW603344, BE748082, W39532, BE543246, AA047141, BF800032, N34692, BE168974, AA340956, BE620236, BE937574, BE122763, AW818992, AA304405, AF193611.1, BC001278.1, AF015287.1, AL136914. 1.</p>
HPEBA84	78	753957	1 - 1146	15 - 1160	<p>AV702197, AW964983, AI283912, AV688589, AA679863, R77836, AA112690, AI686145, AA342049, AA658167, AA112689, AA640275, AA342050, BF876992, AL161799. 19.</p>
HSAVA08	79	580870	1 - 1047	15 - 1061	<p>AA523633, BE562634, AI828787, AC008738.6, AC005722.1, AC020908.6, AC090942.1, AL035685.21, AL049843.18, AC027124.4, AC005089.2, AC084865.2, AC002465.1, AC034251.5, AC022211.5, AL050335.32, AC009123.6, AC005320.1, AC002365.1, U91323.1, AJ251973.1, Z95115.1, AL359792.3, AL133545.10, AC011444.5, Z95152.1, AC002378.1, AL139352.16, AL122035.6, AC006160.9, AL109825.23, AC005015.2, AL162430.15, AL033526.24, AP000697.1, AC005328.1, AC007907.2, AL353653.19, AC010463.6, AC007637.9, AL161779.32, AC008755.6, AC018644.6, AC002996.1, AP001712.1, AC005756.1, AC010363.6, AC005225.2, AC002544.1, AC002470.17, AC003684.1, AC011480.3, AC007277.2, AC010878.4, AC011491.5, AL022394.3, AC068799.14, AL137918.4, AP001726.1, AC006130.1, AL138827.16, AC073864.28, AL034420. 16.</p>
HSHAX04	80	812178	1 - 1273	15 - 1287	<p>AL518245, AL513560, AL518244, AL527318, BE796445, BE729339, BE794797, BE729215, BF570366, BF969527, BE513213, BE797077, BE409736, AV715789, BE729781, BG165883, BF569872, BE730249, BF025786, BF971834, BE747502, BF974060, BF127469, BE394313, AI192460, BE277674, BE387113, BE408890, BF243019, BF685280, BE252479, BE274070, BE386787, BF686552, BE408335, BG179144, BG253243, AI193657, BE383115, BF109402, BE727996, AI978927, BE747511, BE313903, BF027209, BE394458, BG167026, BE387005, BE257613, BE395496, BF127655, AI819133, BE408659, AV701134, BF975755, BE297737, BE276425, BE257900, AW467999, BE409043, BF129135, BE621412, BE251025, BF132821, AV701355, BE736137, BF684777, AA151920, BF205110, AA476567, BE393897, AI589910, BE393107, BE266145, BE390224, BF132684, AA746035, AW406080, BF438288, BE745519, BF238211, BE258291, BE385352, AA224497, BE789268, BF237992, AV701339, AA476450, BE389845, AA922493, AA058904, AV712372, AA809660, AI972258, BE390228, AA031495, AI128546, AA033810, AA934757, AA031586, AI167713, BG111292, BE266333, AV736079, BE294483, BF132501, AI086942, AL513559, AI749671, AA904234, BE410720, AW069373,</p>

					<p>AA535057, BF835180, BF000188, AA224434, BF591243, AA037753, BF856066, AI189102, AA338222, AI284100, AI198266, BF239384, BF834463, H45086, AI079772, AI620932, W87609, C75448, AI498905, BE621931, AW272369, C75433, H45164, T68206, C75434, AA039244, AW473012, C75456, AA043857, AA627969, AA961372, AW192043, BF759521, AA643805, BE048627, AA916520, AU117291, AA327300, BF836781, AI247228, BF836803, AA480241, AA768298, AI187734, BC004898.1, BC008451.1, AF104012.1, AF042385.1, AF042386.1, AF104013.1, AL049824.4, AL033526.24, T57534, H45163, N79482, W87900.</p>
HSKDR27	81	580874	1 - 748	15 - 762	<p>AI984221, AI740960, AW015044, BF591015, W80440, AI141908, AA627626, AA969950, AI581286, AW613262, AW170703, AW073992, BF338322, AA480836, R52038, BF436470, AW074677, AA994760, BE219883, AI381244, BE677262, R52037, BE327304, R49984, AW304136, BE042923, R47846, AA359428, AI538725, AW836013, C00374, BE550516, AA887620, AW873686, BG107838, BE048302, H50794, AI961625, W79036, AF177941. 1.</p>
HSQBF66	82	560726	1 - 1010	15 - 1024	<p>AL043876, D44625, BE674949, AA573644, AV652267, AA579352, AA745570, AA579353, AI732225, AA085683, AL356791.9, AL031985.10, AC006452.4, AC022007.3, Z68885.1, AL096838.1, AL513131.1, AL121890.34, AC026172.3, AL135911.16, AL122035.6, AP001208.3, AP000907.5, S83170.1, AC024163.2, AC025438.5, AC091118.2, AC002299.1, AL049795.20, AL161449.7, AC004458.1, AC000118.1, AL031662.26, AP001537.1, AC010530.7, AL163218.2, AC007676.19, AC005098.2, AF235097.1, AL035690. 10.</p>
HSRFD18	83	840771	1 - 1875	15 - 1889	<p>AL037444, BE889424, BF439488, BF446681, AW297772, BE644962, AW295194, BF740081, BF813145, BE466328, AA716418, BE675824, BF196138, AI280149, AA814882, AA813632, BE221432, BE677733, AA358821, BF246362, BF700198, AW452195, AU144490, BE220601, AA761963, AA658222, AA327922, AI123037, AI473112, AW390382, BF992291, BE928864, R99503, AA055144, AU119961, AA182647, AW390381, AU130604, AA182483, AW390436, BF693283, AA054934, BF849246, R98214, AU117739, BF993158, BF002983, AA248903, AA179387, AA182760, AA934941, AI474620, BF670121, BF858341, BE928859, BE150427, AI912621, AA938230, BF919684, AF288393.1, AL096819.17, AK023095.1, AK000945.1, AL117441. 1.</p>
HSWBE76	84	751308	1 - 860	15 - 874	<p>BE620901, BG170181, BE620502, BE905496, AA195064, AI674742, AU150515, BE222944, BE965160, AW071814, AU147333, AW081850, AU148556, AI433777, AA708102, AI625507, AU150042, AI333540, AI022464, AW024603, AI423210, AA195011, AA708100, AU160595, BF939994, AI365587, AI268519, AI540265, AI285640, AA252209, AA581561, AA913601, AA603763, AA009729, AA009444, AA252208, AA886783, AA724048, AW391826, AI079718, AA775594, R48504, R48503, AA427500, AW881973, BE005990, AA405752, AA430679, BE005985, AW265644, AI424413, AW275442, BF764539, BG251413, AI890866, BF899981, AV726282, AI207963, AI216407, AI797190, AA862943, AA398675, AA393321, AA599014, AI097301, AI279255, AV752744, BE349244, AU150691, AW068586, AW628679, AI003284, AW780020, BF994241, RI2340, H09530, AI612829, AV649791, AW887050, W45434, AK001237.1,</p>

					<p>AC079175.24, AL392044.7, AC002539.1, AC002538.1, AC090526.3, AF235098.1, AF129077.2, AC006117.1, AL357503.12, AC004882.2, AC006379.2, AL096770.16, AC005010.2, AL355977.11, AC061709.25, AL034451.26, AL133467.4, AL033329.25, AL031386.1, AC002086.1, AF165147.1, AP001727.1, AL139389.16, AL163151.1, AC053466.5, AC005529.7, AF165175.2, AC073068.8, AL583784.3, AL357150.7, AL163247.2, AC016254.14, AC013471.7, AL161935.10, AC009753.5, AC005023.1, AC004456.1, AC009949.9, AL049713.20, AC003986.1, AP001671.1, AL096776.12, AC020908.6, AC018468.4.</p>
HT3BF49	85	838620	1 - 2160	15 - 2174	<p>AW450103, AI286250, HI5073, HI5072, AI283763, AW451893, AL355304. 12.</p>
HTEEW69	86	764835	1 - 1268	15 - 1282	<p>BE253978, BE254398, BE781341, BE255799, BE780436, BE780457, BE255033, BE251940, BE257706, BE780314, BE783528, BE255909, AA887084, AW172618, BE253356, BE257391, BE257100, AA913157, BE252558, BE256521, BE618088, BE258350, BE253421, BE251745, BE252959, AI184620, AI024872, AI581295, AI024850, BE256106, BE778121, AA062589, BE255962, AA938866, BE251245, BE259105, AA953444, BE258660, T19332, AI351056, AA700997, BE251072, BE259435, AA063062, AI016246, AA406443, AA994466, T36111, AI017555, AW025700, AA364302, BE259217, AA729497, BE255046, AW966401, U25928, BE251141, AA410460, AA776786, AV702417, AV695700, AV729376, AW950211, AV703687, AV706417, AV709092, AV686100, AV696754, AW952007, AW956891, AW964251, AW960655, AV728652, AV705299, AV705340, AV702296, AV726787, AW951270, AV729076, AV702998, AW962386, AW949478, AV725153, AV651897, AW962978, AW950443, AV703790, AW960601, AW952403, AW952183, AW959806, AV656903, AV661704, AW952751, AV697196, AW956075, AV645936, AV709587, AW955723, AV705135, AV658084, AW959980, AV650283, AV692600, AV650315, AW963768, AV659389, AV650591, AV697880, AV727613, AV656373, AV726010, AW964440, AV655280, AV660258, AV708109, AW959521, AV647789, AW956474, AV659294, AV727787, AV650691, AV703146, AV686060, AV725745, AV686064, AW951239, AV660608, AV728148, AV659322, AV726156, AV650768, AV654908, AV726590, AV698545, AV656478, AW959988, AV709314, AV653353, AV708381, AV654070, AV660728, AW951437, AV691080, AW951281, AV702385, AV658275, AW949802, AV652001, AV651955, AW955662, AV707979, AV703669, AV709580, AV727003, AV725208, AV685536, AV725582, AV708786, AW957517, AV659547, AW959543, AV727526, AV703169, AV725618, AV651920, AW954439, AV727510, AV725633, AV725031, AV728518, AV702266, AV725577, AV725033, AV706223, AV728924, AV725617, AW954206, AW960207, AW955900, AV707863, AV725991, AV696931, AW964421, AW952410, AV703062, AV727822, AV699089, AW964410, AV701874, AW950888, AV703501, AW962444, AW964409, AW964585, AW953574, AV702772, AV704774, AW952460, AW955710, AW954237, AV701180, AV651519, AV707401, AV701183, AV658751, AV683669, AV709660, AV704585, AV709935, AV654035, AV707652, AV707663, AV707654, AV728721, AV683994, AV706854, AV704042, AV654282, AV709880, AV697288, AV729220, AW954134, AV698290, AV687035, AV704847, AV694836, AV706882, AV697498, AV702954, AV655096, AV686420,</p>

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HTEHU59	87	840385	1 - 1509	15 - 1523	BG249175, AL046260, AW963943, AI828967, AW955696, AI760208, AW297718, AI032354, AA417102, AL121147, AA806454, BF216179, AW514160, AA417206, W73366, N39447, N75616, N22370, W73427, N65972, AI370121, AI440453, CI6433, AW470432, N98313, AW204089, AA444140, AL046261, H06227, AI796882, AA443958, AA789252, AI221678, AA716720, AW439530, AW873326, AI143241, N35303, AW613664, AA887889, AA845989, AW665345, BE218168, AI333474, AI333483, AI361785, BE464766, BE326250, AI680844, AA936826, BE348681, N94234, T90568, AI693650, AI971432, AI864735, R32990, AA379582, R40841, AA037360, R79419, AA364460, AW502585, T26572, BF248365, R79420, AA765052, BE763303, AV710415, AW137034, BF916703, N48559, BE504072, AW500077, AA663027, H38490, BE545425, AA953152, AI963960, BE907434, AL049814.6, AL137010. 4.
HTEMQ17	88	840387	1 - 1754	15 - 1768	BG255431, AW365828, BF031371, AW365643, BF107518, BF211270, AL530660, AL120983, AW365846, BE141392, AI276663, BG121604, BF857983, AW365821, AW197389, BE141378, AW504995, BE141375, AV713095, BF811334, BE885456, AI683415, AA758267, AA332445, BF095659, BE152545, AA298292, BF695980, BE152546, AA319133, BE152543, AL040847, AA337156, AK027539.1, Z69890.1, AE006462. 1.
HTGBK95	89	834490	1 - 1117	15 - 1131	AL522125, AL522126, AL519350, AL529333, BF978108, BE217821, AW299468, BG106602, BE326803, AU149173, BE646271, AU153293, AU154281, AI432448, AI039818, BE467032, BE042910, AU152129, AU160794, AU153911, AI571337, AA237091, AI963695, AI635374, AA932292, AW043706, AI302679, AA236679, AA767544, AI735388, N42646, AI590210, AI224546, AI632813, AA234900, BF338277, AI085872, AW002721, AI049665, AU149933, AI142800, AI269171, AW242940, AI741857, BF034584, AI653576, R94321, N68116, AA513076, R43971, R94225, H05324, H24266, BG111524, R97540, Z41226, AA235171, N67392, AA991730, BF925392, AI521589, BF966823, BG110768, AW410784, BE205895, AI914747, AV699173, AV752994, BF037819, AL046227, AP001728.1, AP00010.2, AP001432.1, AP000151.1, D87343.1, AC007383.4, AP000344.1, AC007172.6, AL021155.1, AC011481.4, AC006994.4, AL591104.2, AC011491.5, AC002565.1, AC002073.1, AP000030.1, AL512347.14, AC018639.8, AC010092.4, AL391241.21, AC004638.1, AL136168.4, AC084865.2, AP001746.1, BC008280.1, AL354760.11, AL132768.15, AL035458.35, AL365338.17, AP001711.1, AK025798.1, AC009779.18, AC015982.9, AL031726.22, AL049539.21, AC004797.1, AC034240.4, AP000208.1, AP000130.1, AL031733.3, AP000247.1, AC083871.2, AC083884.6, AF042090.1, AC018719.4, AC006334.3, AC011811.42, AC007563.2, AC004878.2, AC004227.1, AC020552.4, AP000045.1, AP000113.1, AL118520.26, AC008551.5, AC018758.2, AL163282.2, AL353716.18, AL157372.18, AL590037.7, AP001710.1, Z82214.23, AC008507.8, AC023880.5, AC020928.6, AC008755.6, AL356747.18, AL445645.10, AL031727.42, AC005562.1, AC002425.1, AL079342.17, AL136126.34, AC004883.2, AL034374.2,

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HTLEM16	90	779133	1 - 1901	.15 - 1915	AL537268, AL524867, AL521379, AL524655, AL521380, AL520082, AL528768, AL513950, AL527410, AL532992, AL518562, AL526411, AL520081, AL524866, AL527368, AL518561, BF793507, BE293505, BE797874, BF966727, BF793437, BE293461, BF688814, BF690146, BF966760, BF340717, BF663834, AL524654, AA781166, BF342274, BG179677, BF570071, BF663178, AL537267, BF690560, BE903323, AV728729, BF340891, BF528974, BE257966, BE278858, BF515895, AW964631, BE297161, BF968582, BF026117, BE733780, BE280977, BF724855, BE312997, AA641020, BF529180, AL519567, BE279987, BF344151, BE255018, BE390139, BF701583, BF688333, BF966530, BF345769, BE302964, BF345255, BF569584, BF206038, BE249931, BF026959, BF966315, BE257931, BE730892, BF061136, AA621730, BF058285, BE900610, AL526371, BF347440, BE302621, AW131766, BF529014, BF984577, AI803361, BF439974, BF527493, BF967158, AI708896, BE900258, AW131694, W21824, BE408464, BE563677, BE251526, BG024544, BE780162, BE257745, BE892373, BE727832, BE727561, AW026308, BG035433, AL513949, BE731124, BE896130, BE276768, BE385541, BF240238, BF984799, BF689920, BE390488, BE778942, BF084737, BF965298, BE905202, AA640946, BE383842, BF724132, BG024277, AI279215, BF529777, BE314506, AW167695, BE407700, AW027751, BE904285, BE384005, BF027626, BG031193, BG106859, BF725279, AA044161, BG025646, BG119068, BF346642, BF027086, AL134384, AI299018, BE281345, AA908781, AA496423, H38040, AI095564, BE906042, BF026948, BE408496, BF528680, AI338106, W22099, BF111929, BF689664, AA086051, AI138962, AI815598, BE895892, AA921766, AA809477, BF851430, N95209, AA402419, BE302043, BF026902, AA284506, BF446741, BE547438, BG056414, AA761749, AW152609, AA679123, AA526535, N78612, AA809563, W27812, AA903910, D52750, H41405, C15929, BG104603, N70493, H15374, H15378, AI033087, BE764253, BF670047, AV648334, AI871263, T33274, R44660, AW513845, AA044069, BG059536, BE350072, AW166762, BE207476, AW262785, AA961219, AW190491, T30636, AW195111, AV723609, BE731711, W04862, BF203872, BE207570, H73593, AA527956, H41532, AI813904, BF340463, BF984596, BE249854, AW873291, AA976134, BE870399, R59073, AI301136, R59953, BF724545, BF434606, AI341591, BF795344, Z43745, AW006965, AI539828, AI620455, R71196, AA262408, H38431, AA746078, BE300768, AA768657, AA287226, H39122, AI688746, R59074, AW968070, AI221081, H45573, BG036289, BF732285, R89743, R52105, R89524, AW192972,

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HTNBK13	91	831967	1 - 1146	15 - 1160		<p>BE799670, BE794458, BF969839, BF116235, BE894258, AI755110, BE693669, AA209372, AA209368, AV702645, AW957276, AV724122, AW517214, AW173346, AA197278, AI609300, BF726226, AI261762, BE882052, AI400083, AA112077, AI242204, AA114827, AA314213, AI741473, AI828740, AI982748, AA197243, AI140451, BF923463, AA838629, AA854805, AA114846, N59363, AA931373, AA972617, BF358017, AI687104, AA234016, AA843577, AA625125, AA133768, AA911212, AI553981, AA304885, AA133767, R76792, AI559186, AW874604, BF358016, BE180724, AA486696, BG248840, AA733214, R75956, H24319, AA447346, AW839247, AA633116, AI187039, AA877750, AA938362, AA083910, AA384854, N32060, H23039, AA678500, F16874, AA972329, T84723, AW372424, AW372436, AA906710, AW372421, AW372425, AW372444, AW372435, AW372434, AW372427, AW372439, BF897466, AW372423, AW062891, AW372443, AW372420, AW372440, AW372433, AW372437, AW372422, AW372432, AW372442, AW372430, AW372428, AW372419, AW372429, AI383049, AI383050, AW372431, AI702272, AI701769, AW372274, AW372408, AA092723, AA634344, AW805484, AI623944, AI985597, AW073164, AW372407, AA352209, AW749114, AW629894, AA912615, AA825365, AA029876, H55702, BE814640, AA975153, T52666, AI540674, AW073898, AA857847, AA808175, AV721824, AI298026, AW834282, AW952443, AI287233, AI784233, BE966278, AA809897, AL514721, AI698391, AI500714, BE905211, AI978703, BE874163, AA868961, AW051088, AL514025, AI570884, AI250627, AW827249, AI366900, AV713528, AI633125, AI538564, AI915291, AW152182, BE393784, BE843239, AI582932, AI590043, BF970040, BF811804, AI889189, AL514511, BF724894, AA641818, AI866469, BE965208, AI884318, AI638644, AI570056, W74529, AI804983, AL513779, BF814449, AI699823, AI345745, AL514871, AV733582, AI351959, BE896769, AI628337, AL513999, BG026483, AI610714, AW189332, AV726590, BF811802, AI538885, AI274507, AI423198, AV753074, AL043070, AI521005, AI621341, BG029667, AI571966, AI539690, AI500061, AI702527, AI741158, AW020693, BG105241, AI927233, AI979129, AL514279, BF970768, AI670002, BE881211, BE964961, BE963575, BE967219, AA928539, AV712722, BF089679, AI559524, BE964506, AI583558, AI499570, AW020095, BE784335, AL514455, AI811603, BF750875, AI473536, AL513911, AI922215, AL041150, N33175, AL515033, R41605, AW072349, BF812426, R32821, AV726058, AL513631,</p>

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				AC009079.4.
HTODN35	92	570901	1 - 1580	15 - 1594
HTPDU17	93	840596	1 - 2064	15 - 2078
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HTTDN24	94	766485	1 - 1978	15 - 1992	<p>AL513751, BG035744, BE792734, BE535814, AI968100, AI638191, AI962526, AW590203, AW593447, AW590212, BE541446, BE220296, AA205663, BE564856, BF769037, AI968771, AI632511, AW250856, AA205775, AA205709, BF843264, AA773929, N53214, AA486579, N30623, AI655137, AW014522, BE564422, AI742717, BF028977, BE672098, AA777617, AI146844, AW577576, AI159827, AA563660, BF027920, AI637620, AI078792, AI990191, BF062933, BF062045, AW044164, BF770158, AA256059, N32225, BF769030, BF769032, BF445417, AA486679, AW959397, W22630, AA554325, R59984, AW006249, AW236554, AW577601, AA765281, AI653704, AI206329, R67166, AW243537, AA205674, H00645, T16860, AA256068, N57321, H00733, N41868, T16861, AI149479, AI906144, N59086, BF926652, AA909318, AW594490, AA299517, N58615, AA641283, AA644247, AW236428, AI820831, BG009693, BF851615, AA743510, Z42234, AA834460, AA548119, AI672532, F07004, Z40114, F01762, AA301407, BF085945, BF931293, AA205611, AI638784, BF085928, AA983380, BF059602, AW192770, BE714630, AA205702, AI078796, BC001039.1, AF310106.1, BC003592.1, AK000784.1, AK025952.1, AF298547.1, AK026387. 1.</p>
HTTEE41	95	840950	1 - 1959	15 - 1973	<p>AL533251, AL514520, AL535565, AL519250, AU120401, AL514519, AL513606, AL517678, AI986262, AU139509, AU138912, BF797374, AU124362, AV714807, AI970836, T25350, BG169633, AU126517, BG031251, AA854925, AI683290, AI084631, AU130264, BE748699, AU124315, AU133858, BG109529, AU135232, AI080278, BG119840, AI114754, BG258768, BG255494, BE786284, AI955296, AU126258, BG254492, BF057590, AI342485, BE907879, AV689488, BG035949, AV688329, BE780740, AI982815, BE897302, AL037847, BE619295, BF037149, BE547405, BE540421, BE870861, BG119545, BE891546, BE872237, BG179989, BE784107, AI992184, AU128577, BE893297, BE798745, AI858401, BE871280, BE882225, AI801143, BE748021, BG033360, BF984307, BF795054, BE535364, BE870845, AV711098, BG115930, BE787681, BE542328, BE872802, BF700048, BF695387, AI674907, BE439607, BF984686, AW079041, BG166631, BE896320, BE541060, BG168465, BF212903, BG165234, BF036631, AW276472, AI469127, BG116642, AW304879, BG035549, BE298292, BE383409, AW951669, BE789205, AA876301, AI559157, BE618039, BE569054, BE884211, AA314410, AL037869, BF034922, BG261406, BE278215, AI567289, AI216294, BE536033, AI160646, BE541100, AI652229, BF793118, AA573870, BE781061, AW675729, AV717435, AW967121, AW268555, AW614767, BE871948, BF246592, AW302409, BG231674, AL519249, AI623915,</p>

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HTXJD85	96	840391	1 - 1103	15 - 1117	BF511613, R16403, H06356, AC005874.3, AF134471.1, AC018828.3, AC022383.3, AC005261.1, AL031588.1, AL132653.22, AC008649.6, AP000689.1, AB003151.1, AC005080.2, Z94056.1, AF243527.1, AC011308.8, AC016637.6, AL355074.5, AL391259.15, AC018808.4, AL355543.13, AC034193.4, AC008569.6, AC001231.2, AL354720. 14.
HUVDI48	97	564853	1 - 1813	15 - 1827	AI479925, AV720735, AI886110, AF261918.1, AB037733. 1.
HWBBU75	98	780360	1 - 2717	15 - 2731	BG254332, BG258993, AW189216, AW189167, BG110226, AA621650, AI524238, AW027302, AI085248, AI187104, AJ403112, AW129561, AA287508, AA287381, AI569606, AW137239, AI814239, BE246677, BF356218, H87267, AI831754, H87322, W15561, AA831701, AA808000, BE086121, AW137386, AA932127, H92853, BF916818, R84247, BE672348, BF869463, AI380578, BE696648, N80792, AA811717, AA922507, BF945407, AK024474. 1.
HWHPB78	99	740778	1 - 1332	15 - 1346	AA004226, AA007259, AW071800, AW337233, BF684823, BE560744, AU143103, BF981277, AL528300, BE890251, AL525528, BE561304, AL534641, AW812566, AI198256, AL523832, .

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HWLBO67	100	834315	1 - 522	15 - 536	AC011739.7.
HWLGP26	101	834770	1 - 1884	15 - 1898	AL529987, AV699741, BF530871, AW207742, AI638032, BE540656, AI469103, AW575118, BE503442, AI652512, AA928729, AW592532, AI419960, AI057140, BF102699, AI365238, AI392687, N49757, AA832314, BF819685, AI025113, AI767982, AA766124, AU151363, AW402473, AI654109, AW070683, AI738949, AA815032, BG231904, AW578625, AI365240, AV688777, AA769270, AW613566, AA605050, AI459543, AI885560, AW361738, AV689923, AA046663, BE242932, BF895261, AA507121, AA046576, AI627311, AW296010, AA814924, BG253328, BE789823, BE243489, AA298793, AI458707, AI208523, AA694217, BE247085, AW137352, AV689313, AA077726, BE241524, AL523598, AV685517, BE241950, AV697781, AV691130, AV688018, AV697631, N52685, AW182301, AA910584, AA126044, AW390687, AL529988, AF176097.1, AK023002.1, AU131891. 2.
HILCA24	102	869856	1 - 1968	15 - 1982	BE780749, AU137314, AV732875, AW954734, AW138881, BF681107, AI079555, AI624252, AA233208, AU157126, AI734898, AW088851, BE221267, AA314962, AV715966, AA971982, AA233124, AA129416, AA133798, AA886808, AA353195, AW132033, T98200, H50558, AI888751, AI818363, BF917932, BF926224, AI784628, H50559, T98201, AK001989.1, AL512750.1, AC010627.5, AC010491.3, AC026749.5, AC016656.5, AC016652. 5.
HE2CA60	103	888705	1 - 3020	15 - 3034	AL535023, AL514917, AV727366, AW960741, BF970019, AV711225, AV658809, AV721596, AV658785, AU126984, AV693513, BF129964, AI140773, BF576888, BF929570, BF102505, BF677857, BF697387, AW006994, BF448157, AI302186, AW770389, AI888667, AA215792, AI337827, BF701132, AI573244, AW173639, AI870916, AI635189, AI678655, AI379341, AV748873, AI417164, AI023944, AW769389, AW021198, N39649, AA311424, AA593881, BF693869, AI538428, BF343015, BE350890, AA527258, AA446972, BF693703, AI673388, AV725444, AI160026, AW166928, AW084026, AI246492, AV728808, AI865154, AA535263, AA278438, AW958219, AI598040, N50839, AV703798, AV726287, AI567526, AA568228, AA780953, N90778, AW467601, AA411969, AI038481, H27220, AW296240, AA253481, W93347, AA232931, BE439630, AA932649, BF914259, AA716259, AW796151, AI080112, AI673834, AU156818, AV655775, AW190288, AA234891, AI953472, AA814625, AU158056, AI289270, AI702014, AI908123, AU154032, BE813629, AI446206, AA075083, AA232943, AI908087, AA621767, AI288409, AI961349, AA075001, AI470257, AA573515, AV686052, AV683900,

HPWTF23	104	844775	1 - 1994	15 - 2008	<p> AV708123, AA906650, AA598599, N75631, N22393, R87834, H28574, C20658, BF214864, BE089894, BE089889, BE089884, BE568741, BE177263, AW103250, AI567153, AA029909, AV707840, AI277579, N66892, DI11764, AA588488, AV713215, AI091052, AA760879, AA451779, AA436604, AA449973, AW952511, AA743486, H88193, AA761104, AA887248, AI537734, Z28798, AI868443, H27622, AA460194, BE769290, BF213107, AA215630, AW369729, AV748115, AI630957, AI217501, BE765338, BE243642, N49735, AA030038, AL535022, AW973619, BE715613, AU119491, BE715618, T32443, R41621, AI908149, R18666, D62030, BE715610, BE715622, BF240978, AI492659, AV721162, BE715623, AA412738, AI929527, AI870127, BF155088, AA337914, AI886159, AI880332, R87759, AA578074, BF347940, C03599, AW149323, AI858885, AW582368, BE715600, R23365, AA503713, N56115, AA861658, AA429509, AW937431, AV712224, AI587356, AA279034, AA730462, AA366526, AA331496, BE769848, AW582361, AI908105, BF198101, BE768029, AI525041, AI566976, AA252936, BE167506, AA337416, H88194, AW805194, Z28797, AA375774, AA337493, AA304794, N26583, AA248761, AA336736, AA918009, N86808, BE768102, N56183, BF813368, AA332577, AW360775, BF699012, AW607859, AA037239, AA234184, AW385045, R94890, BE768193, AA248808, AA377361, AI679117, N87842, BF208805, AW610530, R58320, BE172045, AA218663, AW472993, BF448490, AA587998, AA668344, T55233, AW089673, N54694, AI950073, BF240775, AA995161, AB034205.1, AC005921.3, AK027723.1, AL050121.1, AF069250.1, AK023672.1, AK001925.1, AF147357.1, AC007383.4, AC006336.4, AL161899.21, AC019159.8, U95739.1, AL355834.4, AP001699.1, AC006994.4, AC016652.5, BC000799.1, BC006832.1, BC006103.1, AL449305.4, AC012502.3, AC006373.3, AC019176.4, AC005291.1, AP001623.1, AL133325.20, AL355512.22, AC012368.6, AC010088.3, AC004383.1, AL360294.11, AC007298.17, AC066585.5, AP001746.1, AL133258.16, AC026431.3, AC010319.7, AL136850.1, AP000130.1, AP000208.1, AF124728.1, AL121656.2, AC022402.4, AL133665.1, AP000247.1, AC016144.13, AK000753.1, AC006039.2, AB050410.1, AC044797.5, AL512733.1, BC008078.1, AC010149.8, AC006222.1, AC024028.10, AC010081.4, AL050309.4, AC068715.5, AC006241.1, AF061943.1, AC010723.3, AC026888.6, AL133405.17, AC025735.4, AL137527.1, AC083867.4, AC006288.1, AL137558.1, AC006338.5, AP001346.1, N52664, AA278736, N88556. </p>
HPWTF23	104	844775	1 - 1994	15 - 2008	<p> AL519322, AL519321, AL528621, AL525472, AV708533, AV707483, BG117131, AV701688, AW954252, AV701690, AL525317, BF968790, AV709370, AV707363, AV657517, AI635895, AL036882, AW857209, AL036883, AV707606, BF128575, BG121441, BE295239, AW392020, BF311431, AV762694, AV706829, AA523541, BE073173, BE295817, BF514386, BE675214, AI949219, AW262884, BE858056, BG055038, BG249916, BG037033, AW371034, AI674706, AI708919, AV715587, AA535070, AA679292, AW022838, N50052, BE830095, AU148045, BE870349, BE349624, BE350028, BF837239, AA147899, BF845844, AW073776, AU147814, BF725674, AI368808, AI927857, AI342463, AV701888, AI140105, AA487093, AV750709, BE858057, AW190710, AA147844, AI096573, AA716187, AA722314, AI862165, W63717, </p>

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HGCAC19	105	851527	1 - 5047	15 - 5061	AL527635, AI582588, BE619956, AW003219, AW195551, AI114573, BE888827, BF444977, AU139098, AA307878, AI915534, AU117064, BE466128, BG251218, BE619434, AI700569, AI823371, BG260998, AI625554, AI478557, AW958690, AI052694, AW963950, AL134475, AU136749, BE326684, AW770747, AI803406, AI990669, BF448130, AW770442, AI193790, AI659257, AV757384, AI669806, BE835313, AI917737, AA947974, BE881668, BE466358, AI698025, BF061334, BF433914, BE551956, AW003148, BF055107, AW237192, BE646481, AW949672, AA910441, AA994008, AA804520, AW058531, AI247672, BE890358, AA156928, AI767577, AW949361, AW166817, BG104926, AI971146, AW299771, AI990108, AI631706, BG114047, AA824598, AA156804, AA312557, AA677603, AI636032, AI400786, BE768656, AI199770, AI989485, AA155855, AI802754, AV650187, AW014065, BF677900, BF680207,

					AA557259, AI925305, AA446690, AI261268, AW150014, AA155958, BE379872, BF215025, AW867051, BE672462, BE168338, AI350222, AI624072, AI825185, AA436183, AA916694, AA436309, AW867047, BE139075, BF530119, AI656311, AV755927, AV746485, AW770099, AA147323, BE768554, AI885519, BE768542, AA446565, AI821288, BE245294, AW129764, AA599626, AW818369, BE245262, AI536551, AI040161, AI446564, AI627777, AA020816, AV758283, D80771, AA806383, AA136672, H40373, BE042467, AA013438, AI591292, AW472897, AI821040, AI820997, AA180321, AI356314, AA312855, AI610014, BF902177, AW195825, C75219, AW015240, AI936012, AI219472, AA086094, AW169820, AW983970, AA113317, AI282736, BE175626, BF061715, AI275943, AW294050, AV738635, AA358726, AA463692, BF896130, AW183617, H21867, BE168125, AI246262, AA059237, AA677761, AA628617, BE768595, AI625290, AA007519, BE768665, AW374169, AA355425, T07209, AA377468, AW271631, AA152212, AW189878, AA086093, AW594290, AA742173, AI949786, AW023249, AW750461, AA256785, BE872264, AA709003, AA147425, AA361135, H40320, AW392416, AW392849, BF081349, AI038854, AW188893, BE763982, AA976161, AA345273, AW858464, AW891017, D52302, AA358727, AI699855, D61215, D52967, AA430996, AW376216, R81478, AW501079, AA007520, AA353267, AA728991, AA304423, AA356393, AI370683, AW024132, T71726, AA361495, AV724116, AI888048, BF844493, T40389, BF089968, T71578, AA621423, AW043940, AA513522, BF763717, R62569, BF950322, AA334984, BF091136, BE892057, AW188923, AA826001, N24122, BE768735, AW382160, BF985865, T18537, BF365430, AI830982, AW452149, AW188842, BE702825, AI637868, AA732593, AA376840, AW858513, BF804806, D19713, AW131746, AA256511, AA829191, AI310882, AF109907.1, L40392.1, AK026107.1, AC004858.2, S67071.1, AF135593.1, AC006345.4, T60745, R25599, R26428, R26995, AA136788, AA150143, AA888518, AI053404, AI053507, AI053899, N64912, N64913, C00711, AA393814, AA905385, AI002840.
HEQBJ01	106	876546	1 - 2777	15 - 2791	AU123703, AI744148, AI744113, AU130720, BE897357, BG105308, AU137281, BF308835, AI860811, AI889014, AI765413, BE546221, BE670583, AW237314, BG248530, AW952369, BE502734, BE503479, AI765401, BE540301, AL042645, BF212478, AW500417, BG249708, AW674190, AI867571, BE018153, AW293518, AA534578, AI432178, AW169762, AA506984, BE389321, AA420605, AI142237, AA406169, AW591668, AW188054, AI147954, AA430324, AL040186, AI197943, AW502601, AI589634, AA569041, AI015938, AA433904, AA070872, AI188829, AI124780, AA421239, AI149224, AA420647, AI916160, W73655, AI076564, AI768356, BF592904, BE464156, AW772442, BE219974, AI638215, BG034042, AI125307, R51293, BE812953, W51790, AA172002, AA425349, AA565222, AA313542, AA825728, R35270, AW204507, BF306159, BG152825, AA100809, W28763, BF223048, AI222042, AI479185, W26572, W45413, W73608, R52192, AI160529, AW440819, AI422286, AI298011, AA171761, AA421279, R51403, H62930, R52097, R59309, BF880022, AA581790, W81419, BF844067, AI768849, W40121, AI708313, AW511347, AA373236, AW368276, BF844000, AA434583, AV747735, Z42217,

					BE702753, W81420, AI962360, AA325784, BF879714, R59310, AI271621, T25845, BF879715, T06069, F05246, BF880008, AA806028, Z38264, AA071023, AA815452, N54389, BE929180, BE929187, BE929175, BF092052, AA810542, AA383377, AI370602, R50941, T87272, BF149192, T87186, AW867815, F01748, AA947741, BF749400, AA773493, AA890049, AW591526, AI985779, AA984284, BF923416, AW272799, AL043147, AB007891.1, BC002701.1, AC009079.4, AL157384.8, AK025058.1, AK026926.1, AC009779. 18.
HBJHT01	107	587262	1 - 1237	15 - 1251	AW572853, AW852662, AW794904, AW852694, AA494184, BE092308, AW467441, BG000611, BF871414, BF871418, AA715850, AW962959, AI281561, BF899671, AI537814, BF899672, BF970033, BF899670, AW975902, BF847493, AW197990, AC084865.2, AL590763.1, AC008403.6, AC002551.1, AP001858.4, AP001692.1, AL450224.1, AL160269.14, AL022476.2, AL049762.20, AC011811.42, AL356020.3, AC078818.19, AC007842.1, AC007055.3, AC008569.6, AC005803.1, AB020865.1, AL353574.8, AL138836.15, AJ277546.2, Z93930.10, AC005484.2, AC069548.4, AC007619.22, L44140.1, AL158040.13, Z95115.1, AF196779.1, AC009155.3, AP003116.2, AC005548.1, AL354696.11, AC005254.1, AL353579.17, AC012323.7, AC007371.16, AL049780.4, AC000353.27, AL022336.1, AC009309.4, AL158830.17, AC005488.2, AL591076.5, AC007954.7, AL031664.1, AC000025.2, AL121586.31, AC004859.2, AL365364.19, AL049757.14, AL390205.17, AC007011.1, AL353812.13, AL157877.11, AP000893.5, AL021391.2, AC087244.17, Y10196.1, AF001549.1, U91323.1, AL096701.14, AC034193.4, AP000501.1, AC022211.5, AC011472.7, AP002852.3, AL031650.22, AC008745.6, AC011005.7, AC006930.1, AF053356.1, AL133174.15, AL137918.4, AP001715.1, AC018808.4, AC009488.5, AC027319.5, AL110115. 38.

Description of Table 4

Table 4 provides a key to the tissue/cell source identifier code disclosed in Table 1B.2, column 5. Column 1 of Table 4 provides the tissue/cell source identifier code disclosed in Table 1B.2, Column 58. Columns 2-5 provide a description of the tissue or cell source. Note that "Description" and "Tissue" sources (i.e. columns 2 and 3) having the prefix "a_" indicates organs, tissues, or cells derived from "adult" sources. Codes corresponding to diseased tissues are indicated in column 6 with the word "disease." The use of the word "disease" in column 6 is non-limiting. The tissue or cell source may be specific (e.g. a neoplasm), or may be disease-associated (e.g., a tissue sample from a normal portion of a diseased organ). Furthermore, tissues and/or cells lacking the "disease" designation may still be derived from sources directly or indirectly involved in a disease state or disorder, and therefore may have a further utility in that disease state or disorder. In numerous cases where the tissue/cell source is a library, column 7 identifies the vector used to generate the library.

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Table 4

Code	Description	Tissue	Organ	Cell Line	Disease	Vector
AR022	a Heart	a Heart				
AR023	a Liver	a Liver				
AR024	a mammary gland	a mammary gland				
AR025	a Prostate	a Prostate				
AR026	a small intestine	a small intestine				
AR027	a Stomach	a Stomach				
AR028	Blood B cells	Blood B cells				
AR029	Blood B cells activated	Blood B cells activated				
AR030	Blood B cells resting	Blood B cells resting				
AR031	Blood T cells activated	Blood T cells activated				
AR032	Blood T cells resting	Blood T cells resting				
AR033	brain	brain				
AR034	breast	breast				
AR035	breast cancer	breast cancer				
AR036	Cell Line CAOV3	Cell Line CAOV3				
AR037	cell line PA-1	cell line PA-1				
AR038	cell line transformed	cell line transformed				
AR039	colon	colon				
AR040	colon (9808co65R)	colon (9808co65R)				
AR041	colon (9809co15)	colon (9809co15)				
AR042	colon cancer	colon cancer				
AR043	colon cancer (9808co64R)	colon cancer (9808co64R)				
AR044	colon cancer 9809co14	colon cancer 9809co14				
AR050	Donor II B Cells 24hrs	Donor II B Cells 24hrs				
AR051	Donor II B Cells 72hrs	Donor II B Cells 72hrs				
AR052	Donor II B-Cells 24 hrs.	Donor II B-Cells 24 hrs.				
AR053	Donor II B-Cells 72hrs	Donor II B-Cells 72hrs				
AR054	Donor II Resting B Cells	Donor II Resting B Cells				
AR055	Heart	Heart				

AR056	Human Lung (clonotech)	Human Lung (clonotech)					
AR057	Human Mammary (clonotech)	Human Mammary (clonotech)					
AR058	Human Thymus (clonotech)	Human Thymus (clonotech)					
AR059	Jurkat (unstimulated)	Jurkat (unstimulated)					
AR060	Kidney	Kidney					
AR061	Liver	Liver					
AR062	Liver (Clonotech)	Liver (Clonotech)					
AR063	Lymphocytes chronic lymphocytic leukaemia	Lymphocytes chronic lymphocytic leukaemia					
AR064	Lymphocytes diffuse large B cell lymphoma	Lymphocytes diffuse large B cell lymphoma					
AR065	Lymphocytes follicular lymphoma	Lymphocytes follicular lymphoma					
AR066	normal breast	normal breast					
AR067	Normal Ovarian (4004901)	Normal Ovarian (4004901)					
AR068	Normal Ovary 9508G045	Normal Ovary 9508G045					
AR069	Normal Ovary 9701G208	Normal Ovary 9701G208					
AR070	Normal Ovary 9806G005	Normal Ovary 9806G005					
AR071	Ovarian Cancer	Ovarian Cancer					
AR072	Ovarian Cancer (9702G001)	Ovarian Cancer (9702G001)					
AR073	Ovarian Cancer (9707G029)	Ovarian Cancer (9707G029)					
AR074	Ovarian Cancer (9804G011)	Ovarian Cancer (9804G011)					
AR075	Ovarian Cancer (9806G019)	Ovarian Cancer (9806G019)					
AR076	Ovarian Cancer (9807G017)	Ovarian Cancer (9807G017)					
AR077	Ovarian Cancer (9809G001)	Ovarian Cancer (9809G001)					

AR078	ovarian cancer 15799	ovarian cancer 15799							
AR079	Ovarian Cancer 17717AID	Ovarian Cancer 17717AID							
AR080	Ovarian Cancer 4004664B1	Ovarian Cancer 4004664B1							
AR081	Ovarian Cancer 4005315A1	Ovarian Cancer 4005315A1							
AR082	ovarian cancer 94127303	ovarian cancer 94127303							
AR083	Ovarian Cancer 96069304	Ovarian Cancer 96069304							
AR084	Ovarian Cancer 9707G029	Ovarian Cancer 9707G029							
AR085	Ovarian Cancer 9807G045	Ovarian Cancer 9807G045							
AR086	ovarian cancer 9809G001	ovarian cancer 9809G001							
AR087	Ovarian Cancer 9905C032RC	Ovarian Cancer 9905C032RC							
AR088	Ovarian cancer 9907 C00 3rd	Ovarian cancer 9907 C00 3rd							
AR089	Prostate	Prostate							
AR090	Prostate (clonotech)	Prostate (clonotech)							
AR091	prostate cancer	prostate cancer							
AR092	prostate cancer #15176	prostate cancer #15176							
AR093	prostate cancer #15509	prostate cancer #15509							
AR094	prostate cancer #15673	prostate cancer #15673							
AR095	Small Intestine (Clontech)	Small Intestine (Clontech)							
AR096	Spleen	Spleen							
AR097	Thymus T cells activated	Thymus T cells activated							
AR098	Thymus T cells resting	Thymus T cells resting							
AR099	Tonsil	Tonsil							
AR100	Tonsil germinal center centroblast	Tonsil germinal center centroblast							
AR101	Tonsil germinal center B cell	Tonsil germinal center B cell							
AR102	Tonsil lymph node	Tonsil lymph node							
AR103	Tonsil memory B cell	Tonsil memory B cell							

AR104	Whole Brain	Whole Brain					
AR105	Xenograft ES-2	Xenograft ES-2					
AR106	Xenograft SW626	Xenograft SW626					
AR119	001: IL-2	001: IL-2					
AR120	001: IL-2.1	001: IL-2.1					
AR121	001: IL-2 b	001: IL-2 b					
AR124	002: Monocytes untreated (1hr)	002: Monocytes untreated (1hr)					
AR125	002: Monocytes untreated (5hrs)	002: Monocytes untreated (5hrs)					
AR126	002: Control.1C	002: Control.1C					
AR127	002: IL2.1C	002: IL2.1C					
AR130	003: Placebo-treated Rat Lacrimal Gland	003: Placebo-treated Rat Lacrimal Gland					
AR131	003: Placebo-treated Rat Submandibular Gland	003: Placebo-treated Rat Submandibular Gland					
AR135	004: Monocytes untreated (5hrs)	004: Monocytes untreated (5hrs)					
AR136	004: Monocytes untreated 1hr	004: Monocytes untreated 1hr					
AR139	005: Placebo (48hrs)	005: Placebo (48hrs)					
AR140	006: pC4 (24hrs)	006: pC4 (24hrs)					
AR141	006: pC4 (48hrs)	006: pC4 (48hrs)					
AR152	007: PHA(1hr)	007: PHA(1hr)					
AR153	007: PHA(6HRS)	007: PHA(6HRS)					
AR154	007: PMA(6hrs)	007: PMA(6hrs)					
AR155	008: 1449 #2	008: 1449 #2					
AR161	01: A - max 24	01: A - max 24					
AR162	01: A - max 26	01: A - max 26					
AR163	01: A - max 30	01: A - max 30					
AR164	01: B - max 24	01: B - max 24					
AR165	01: B - max 26	01: B - max 26					

AR166	01: B - max 30	01: B - max 30					
AR167	1449 Sample	1449 Sample					
AR168	3T3P10 1.0uM insulin	3T3P10 1.0uM insulin					
AR169	3T3P10 10nM Insulin	3T3P10 10nM Insulin					
AR170	3T3P10 10uM insulin	3T3P10 10uM insulin					
AR171	3T3P10 No Insulin	3T3P10 No Insulin					
AR172	3T3P4	3T3P4					
AR173	Adipose (41892)	Adipose (41892)					
AR174	Adipose Diabetic (41611)	Adipose Diabetic (41611)					
AR175	Adipose Diabetic (41661)	Adipose Diabetic (41661)					
AR176	Adipose Diabetic (41689)	Adipose Diabetic (41689)					
AR177	Adipose Diabetic (41706)	Adipose Diabetic (41706)					
AR178	Adipose Diabetic (42352)	Adipose Diabetic (42352)					
AR179	Adipose Diabetic (42366)	Adipose Diabetic (42366)					
AR180	Adipose Diabetic (42452)	Adipose Diabetic (42452)					
AR181	Adipose Diabetic (42491)	Adipose Diabetic (42491)					
AR182	Adipose Normal (41843)	Adipose Normal (41843)					
AR183	Adipose Normal (41893)	Adipose Normal (41893)					
AR184	Adipose Normal (42452)	Adipose Normal (42452)					
AR185	Adrenal Gland	Adrenal Gland					
AR186	Adrenal Gland + Whole Brain	Adrenal Gland + Whole Brain					
AR187	B7(1hr)+ (inverted)	B7(1hr)+ (inverted)					
AR188	Breast (18275A2B)	Breast (18275A2B)					
AR189	Breast (4004199)	Breast (4004199)					
AR190	Breast (4004399)	Breast (4004399)					
AR191	Breast (4004943B7)	Breast (4004943B7)					
AR192	Breast (4005570B1)	Breast (4005570B1)					
AR193	Breast Cancer (4004127A30)	Breast Cancer (4004127A30)					
AR194	Breast Cancer (400443A21)	Breast Cancer (400443A21)					

AR195	Breast Cancer (4004643A2)	Breast Cancer (4004643A2)					
AR196	Breast Cancer (4004710A7)	Breast Cancer (4004710A7)					
AR197	Breast Cancer (4004943A21)	Breast Cancer (4004943A21)					
AR198	Breast Cancer (400553A2)	Breast Cancer (400553A2)					
AR199	Breast Cancer (9805C046R)	Breast Cancer (9805C046R)					
AR200	Breast Cancer (9806C012R)	Breast Cancer (9806C012R)					
AR201	Breast Cancer (ODQ 45913)	Breast Cancer (ODQ 45913)					
AR202	Breast Cancer (ODQ45913)	Breast Cancer (ODQ45913)					
AR203	Breast Cancer (ODQ4591B)	Breast Cancer (ODQ4591B)					
AR204	Colon Cancer (15663)	Colon Cancer (15663)					
AR205	Colon Cancer (4005144A4)	Colon Cancer (4005144A4)					
AR206	Colon Cancer (4005413A4)	Colon Cancer (4005413A4)					
AR207	Colon Cancer (4005570B1)	Colon Cancer (4005570B1)					
AR208	Control RNA #1	Control RNA #1					
AR209	Control RNA #2	Control RNA #2					
AR210	Cultured Preadipocyte (blue)	Cultured Preadipocyte (blue)					
AR211	Cultured Preadipocyte (Red)	Cultured Preadipocyte (Red)					
AR212	Donor II B-Cells 24hrs	Donor II B-Cells 24hrs					
AR213	Donor II Resting B-Cells	Donor II Resting B-Cells					
AR214	H114EP12 10nM Insulin	H114EP12 10nM Insulin					
AR215	H114EP12 (10nM insulin)	H114EP12 (10nM insulin)					

AR216	H114EP12 (2.6ug/ul)	H114EP12 (2.6ug/ul)					
AR217	H114EP12 (3.6ug/ul)	H114EP12 (3.6ug/ul)					
AR218	HUVEC #1	HUVEC #1					
AR219	HUVEC #2	HUVEC #2					
AR221	L6 undiff.	L6 undiff.					
AR222	L6 Undifferentiated	L6 Undifferentiated					
AR223	L6P8 + 10nM Insulin	L6P8 + 10nM Insulin					
AR224	L6P8 + HS	L6P8 + HS					
AR225	L6P8 10nM Insulin	L6P8 10nM Insulin					
AR226	Liver (00-06-A007B)	Liver (00-06-A007B)					
AR227	Liver (96-02-A075)	Liver (96-02-A075)					
AR228	Liver (96-03-A144)	Liver (96-03-A144)					
AR229	Liver (96-04-A138)	Liver (96-04-A138)					
AR230	Liver (97-10-A074B)	Liver (97-10-A074B)					
AR231	Liver (98-09-A242A)	Liver (98-09-A242A)					
AR232	Liver Diabetic (1042)	Liver Diabetic (1042)					
AR233	Liver Diabetic (41616)	Liver Diabetic (41616)					
AR234	Liver Diabetic (41955)	Liver Diabetic (41955)					
AR235	Liver Diabetic (42352R)	Liver Diabetic (42352R)					
AR236	Liver Diabetic (42366)	Liver Diabetic (42366)					
AR237	Liver Diabetic (42483)	Liver Diabetic (42483)					
AR238	Liver Diabetic (42491)	Liver Diabetic (42491)					
AR239	Liver Diabetic (99-09-A281A)	Liver Diabetic (99-09-A281A)					
AR240	Lung	Lung					
AR241	Lung (27270)	Lung (27270)					
AR242	Lung (2727Q)	Lung (2727Q)					
AR243	Lung Cancer (4005116A1)	Lung Cancer (4005116A1)					
AR244	Lung Cancer (4005121A5)	Lung Cancer (4005121A5)					
AR245	Lung Cancer (4005121A5))	Lung Cancer (4005121A5))					
AR246	Lung Cancer (4005340A4)	Lung Cancer (4005340A4)					

AR247	Mammary Gland	Mammary Gland					
AR248	Monocyte (CT)	Monocyte (CT)					
AR249	Monocyte (OCT)	Monocyte (OCT)					
AR250	Monocytes (CT)	Monocytes (CT)					
AR251	Monocytes (INFG 18 hr)	Monocytes (INFG 18 hr)					
AR252	Monocytes (INFG 18hr)	Monocytes (INFG 18hr)					
AR253	Monocytes (INFG 8-11)	Monocytes (INFG 8-11)					
AR254	Monocytes (O CT)	Monocytes (O CT)					
AR255	Muscle (91-01-A105)	Muscle (91-01-A105)					
AR256	Muscle (92-04-A059)	Muscle (92-04-A059)					
AR257	Muscle (97-11-A056d)	Muscle (97-11-A056d)					
AR258	Muscle (99-06-A210A)	Muscle (99-06-A210A)					
AR259	Muscle (99-07-A203B)	Muscle (99-07-A203B)					
AR260	Muscle (99-7-A203B)	Muscle (99-7-A203B)					
AR261	Muscle Diabetic (42352R)	Muscle Diabetic (42352R)					
AR262	Muscle Diabetic (42366)	Muscle Diabetic (42366)					
AR263	NK-19 Control	NK-19 Control					
AR264	NK-19 IL Treated 72hrs	NK-19 IL Treated 72hrs					
AR265	NK-19 UK Treated 72 hrs.	NK-19 UK Treated 72 hrs.					
AR266	Omentum Normal (94-08-B009)	Omentum Normal (94-08-B009)					
AR267	Omentum Normal (97-01-A039A)	Omentum Normal (97-01-A039A)					
AR268	Omentum Normal (97-04-A114C)	Omentum Normal (97-04-A114C)					
AR269	Omentum Normal (97-06-A117C)	Omentum Normal (97-06-A117C)					
AR270	Omentum Normal (97-09-B004C)	Omentum Normal (97-09-B004C)					
AR271	Ovarian Cancer (17717AID)	Ovarian Cancer (17717AID)					
AR272	Ovarian Cancer	Ovarian Cancer					

	(9905C023RC)	(9905C023RC)					
AR273	Ovarian Cancer (9905C032RC)	Ovarian Cancer (9905C032RC)					
AR274	Ovary (9508G045)	Ovary (9508G045)					
AR275	Ovary (9701G208)	Ovary (9701G208)					
AR276	Ovary 9806G005	Ovary 9806G005					
AR277	Pancreas	Pancreas					
AR278	Placebo	Placebo					
AR279	rIL2 Control	rIL2 Control					
AR280	RSS288L	RSS288L					
AR281	RSS288LC	RSS288LC					
AR282	Salivary Gland	Salivary Gland					
AR283	Skeletal Muscle	Skeletal Muscle					
AR284	Skeletal Muscle (91-01-A105)	Skeletal Muscle (91-01-A105)					
AR285	Skeletal Muscle (42180)	Skeletal Muscle (42180)					
AR286	Skeletal Muscle (42386)	Skeletal Muscle (42386)					
AR287	Skeletal Muscle (42461)	Skeletal Muscle (42461)					
AR288	Skeletal Muscle (91-01-A105)	Skeletal Muscle (91-01-A105)					
AR289	Skeletal Muscle (92-04-A059)	Skeletal Muscle (92-04-A059)					
AR290	Skeletal Muscle (96-08-A171)	Skeletal Muscle (96-08-A171)					
AR291	Skeletal Muscle (97-07-A190A)	Skeletal Muscle (97-07-A190A)					
AR292	Skeletal Muscle Diabetic (42352)	Skeletal Muscle Diabetic (42352)					
AR293	Skeletal Muscle Diabetic (42366)	Skeletal Muscle Diabetic (42366)					
AR294	Skeletal Muscle Diabetic (42395)	Skeletal Muscle Diabetic (42395)					
AR295	Skeletal Muscle Diabetic	Skeletal Muscle Diabetic					

	(42483)		(42483)						
AR296	Skeletal Muscle Diabetic (42491)		Skeletal Muscle Diabetic (42491)						
AR297	Skeletal Muscle Diabetic 42352		Skeletal Muscle Diabetic 42352						
AR298	Skeletal Muscle (42461)		Skeletal Muscle (42461)						
AR299	Small Intestine		Small Intestine						
AR300	Stomach		Stomach						
AR301	T-Cell + HDPBQ71.fc 1449 16hrs		T-Cell + HDPBQ71.fc 1449 16hrs						
AR302	T-Cell + HDPBQ71.fc 1449 6hrs		T-Cell + HDPBQ71.fc 1449 6hrs						
AR303	T-Cell + IL2 16hrs		T-Cell + IL2 16hrs						
AR304	T-Cell + IL2 6hrs		T-Cell + IL2 6hrs						
AR306	T-Cell Untreated 16hrs		T-Cell Untreated 16hrs						
AR307	T-Cell Untreated 6hrs		T-Cell Untreated 6hrs						
AR308	T-Cells 24 hours		T-Cells 24 hours						
AR309	T-Cells 24 hrs		T-Cells 24 hrs						
AR310	T-Cells 24 hrs.		T-Cells 24 hrs.						
AR311	T-Cells 24hrs		T-Cells 24hrs						
AR312	T-Cells 4 days		T-Cells 4 days						
AR313	Thymus		Thymus						
AR314	TRE		TRE						
AR315	TREC		TREC						
AR316	Virtual Mixture		Virtual Mixture						
AR317	B lymphocyte,		B lymphocyte,						
AR318	(non-T; non-B)		(non-T; non-B)						
AR326	001 - 293 RNA (Vector Control)		001 - 293 RNA (Vector Control)						
AR327	001: Control		001: Control						
AR328	001: Control.1		001: Control.1						
AR355	Acute Lymphocyte		Acute Lymphocyte Leukemia						

AR389	HepG2 Cell line Buffer 1 hr.	HepG2 Cell line Buffer 1 hr.					
AR390	HepG2 Cell line Buffer 06 hr	HepG2 Cell line Buffer 06 hr					
AR391	HepG2 Cell line Buffer 24 hr.	HepG2 Cell line Buffer 24 hr.					
AR392	HepG2 Cell line Insulin 01 hr.	HepG2 Cell line Insulin 01 hr.					
AR393	HepG2 Cell line Insulin 06 hr.	HepG2 Cell line Insulin 06 hr.					
AR394	HepG2 Cell line Insulin 24 hr.	HepG2 Cell line Insulin 24 hr.					
AR398	HMC-1	HMC-1					
AR399	HMCS	HMCS					
AR400	HMSC	HMSC					
AR401	HUVEC #3	HUVEC #3					
AR402	HUVEC #4	HUVEC #4					
AR404	KIDNEY NORMAL	KIDNEY NORMAL					
AR405	KIDNEY TUMOR	KIDNEY TUMOR					
AR406	KIDNEY TUMOR						
AR407	Lymph Node	Lymph Node					
AR408	Macrophage	Macrophage					
AR409	Megakarioblast	Megakarioblast					
AR410	Monocyte	Monocyte					
AR411	Monocytes	Monocytes					
AR412	Myocardium	Myocardium					
AR413	Myocardium #3	Myocardium #3					
AR414	Myocardium #4	Myocardium #4					
AR415	Myocardium #5	Myocardium #5					
AR416	NK	NK					

AR417	NK cell	NK cell					
AR418	NK cells	NK cells					
AR419	NKYa	NKYa					
AR420	NKYa019	NKYa019					
AR421	Ovary	Ovary					
AR422	Patient #11	Patient #11					
AR423	Peripheral blood	Peripheral blood					
AR424	Primary Adipocytes	Primary Adipocytes					
AR425	Promyeloblast	Promyeloblast					
AR427	RSSWT	RSSWT					
AR428	RSSWTC	RSSWTC					
AR429	SW 480(G1)	SW 480(G1)					
AR430	SW 480(G2)	SW 480(G2)					
AR431	SW 480(G3)	SW 480(G3)					
AR432	SW 480(G4)	SW 480(G4)					
AR433	SW 480(G5)	SW 480(G5)					
AR434	T Lymphoblast	T Lymphoblast					
AR435	T Lymphocyte	T Lymphocyte					
AR436	T-Cell	T-Cell					
AR438	T-Cell,	T-Cell,					
AR439	T-Cells	T-Cells					
AR440	T-lymphoblast	T-lymphoblast					
AR441	Th 1	Th 1					
AR442	Th 2	Th 2					
AR443	Th1	Th1					
AR444	Th2	Th2					
H0002	Human Adult Heart	Human Adult Heart			Heart		Uni-ZAP XR
H0004	Human Adult Spleen	Human Adult Spleen			Spleen		Uni-ZAP XR
H0008	Whole 6 Week Old Embryo						Uni-ZAP XR
H0009	Human Fetal Brain						Uni-ZAP XR
H0011	Human Fetal Kidney	Human Fetal Kidney			Kidney		Uni-ZAP XR

H0012	Human Fetal Kidney	Human Fetal Kidney	Human Fetal Kidney	Kidney			Uni-ZAP XR
H0013	Human 8 Week Whole Embryo	Human 8 Week Old Embryo	Embryo				Uni-ZAP XR
H0014	Human Gall Bladder	Human Gall Bladder	Gall Bladder				Uni-ZAP XR
H0015	Human Gall Bladder, fraction II	Human Gall Bladder	Gall Bladder				Uni-ZAP XR
H0022	Jurkat Cells	Jurkat T-Cell Line					Lambda ZAP II
H0024	Human Fetal Lung III	Human Fetal Lung	Lung				Uni-ZAP XR
H0026	Namalwa Cells	Namalwa B-Cell Line, EBV immortalized					Lambda ZAP II
H0028	Human Old Ovary	Human Old Ovary	Ovary				pBluescript
H0030	Human Placenta						Uni-ZAP XR
H0031	Human Placenta	Human Placenta	Placenta				Uni-ZAP XR
H0032	Human Prostate	Human Prostate	Prostate				Uni-ZAP XR
H0033	Human Pituitary	Human Pituitary					Uni-ZAP XR
H0036	Human Adult Small Intestine	Human Adult Small Intestine	Small Int.				Uni-ZAP XR
H0037	Human Adult Small Intestine	Human Adult Small Intestine	Small Int.				pBluescript
H0038	Human Testes	Human Testes	Testis				Uni-ZAP XR
H0039	Human Pancreas Tumor	Human Pancreas Tumor	Pancreas		disease		Uni-ZAP XR
H0040	Human Testes Tumor	Human Testes Tumor	Testis		disease		Uni-ZAP XR
H0041	Human Fetal Bone	Human Fetal Bone	Bone				Uni-ZAP XR
H0042	Human Adult Pulmonary	Human Adult Pulmonary	Lung				Uni-ZAP XR
H0046	Human Endometrial Tumor	Human Endometrial Tumor	Uterus		disease		Uni-ZAP XR
H0049	Human Fetal Kidney	Human Fetal Kidney	Kidney				Uni-ZAP XR
H0050	Human Fetal Heart	Human Fetal Heart	Heart				Uni-ZAP XR
H0051	Human Hippocampus	Human Hippocampus	Brain				Uni-ZAP XR
H0052	Human Cerebellum	Human Cerebellum	Brain				Uni-ZAP XR
H0053	Human Adult Kidney	Human Adult Kidney	Kidney				Uni-ZAP XR
H0056	Human Umbilical Vein, Endo. remake	Human Umbilical Vein Endothelial Cells	Umbilical vein				Uni-ZAP XR

H0057	Human Fetal Spleen	Human Uterine Cancer	Uterus			Uni-ZAP XR
H0059	Human Uterine Cancer	Human Uterine Cancer			disease	Lambda ZAP II
H0060	Human Macrophage	Human Macrophage	Blood	Cell Line		pBluescript
H0061	Human Macrophage	Human Macrophage	Blood	Cell Line		pBluescript
H0063	Human Thymus	Human Thymus	Thymus			Uni-ZAP XR
H0068	Human Skin Tumor	Human Skin Tumor	Skin		disease	Uni-ZAP XR
H0069	Human Activated T-Cells	Activated T-Cells	Blood	Cell Line		Uni-ZAP XR
H0071	Human Infant Adrenal Gland	Human Infant Adrenal Gland	Adrenal gland			Uni-ZAP XR
H0075	Human Activated T-Cells (II)	Activated T-Cells	Blood	Cell Line		Uni-ZAP XR
H0081	Human Fetal Epithelium (Skin)	Human Fetal Skin	Skin			Uni-ZAP XR
H0083	HUMAN JURKAT MEMBRANE BOUND POLYSOMES	Jurkat Cells				Uni-ZAP XR
H0085	Human Colon	Human Colon				Lambda ZAP II
H0086	Human epithelioid sarcoma	Epithelioid Sarcoma, muscle	Sk Muscle		disease	Uni-ZAP XR
H0087	Human Thymus	Human Thymus				pBluescript
H0090	Human T-Cell Lymphoma	T-Cell Lymphoma	T-Cell		disease	Uni-ZAP XR
H0095	Human Greater Omentum, RNA Remake	Human Greater Omentum	peritoneum			Uni-ZAP XR
H0099	Human Lung Cancer, subtracted	Human Lung Cancer	Lung			pBluescript
H0100	Human Whole Six Week Old Embryo	Human Whole Six Week Old Embryo	Embryo			Uni-ZAP XR
H0109	Human Macrophage, subtracted	Macrophage	Blood	Cell Line		pBluescript
H0111	Human Placenta, subtracted	Human Placenta	Placenta			pBluescript
H0122	Human Adult Skeletal Muscle	Human Skeletal Muscle	Sk Muscle			Uni-ZAP XR
H0123	Human Fetal Dura Mater	Human Fetal Dura Mater	Brain			Uni-ZAP XR

H0124	Human Rhabdomyosarcoma	Human Rhabdomyosarcoma	Sk Muscle		disease	Uni-ZAP XR
H0125	Cem cells cyclohexamide treated	Cyclohexamide Treated Cem, Jurkat, Raji, and Supt	Blood	Cell Line		Uni-ZAP XR
H0130	LNCAP untreated	LNCAP Cell Line	Prostate	Cell Line		Uni-ZAP XR
H0134	Raji Cells, cyclohexamide treated	Cyclohexamide Treated Cem, Jurkat, Raji, and Supt	Blood	Cell Line		Uni-ZAP XR
H0135	Human Synovial Sarcoma	Human Synovial Sarcoma	Synovium			Uni-ZAP XR
H0136	Supt Cells, cyclohexamide treated	Cyclohexamide Treated Cem, Jurkat, Raji, and Supt	Blood	Cell Line		Uni-ZAP XR
H0140	Activated T-Cells, 8 hrs.	Activated T-Cells	Blood	Cell Line		Uni-ZAP XR
H0144	Nine Week Old Early Stage Human	9 Wk Old Early Stage Human	Embryo			Uni-ZAP XR
H0150	Human Epididymus	Epididymis	Testis			Uni-ZAP XR
H0151	Early Stage Human Liver	Human Fetal Liver	Liver			Uni-ZAP XR
H0156	Human Adrenal Gland Tumor	Human Adrenal Gland Tumor	Adrenal Gland		disease	Uni-ZAP XR
H0159	Activated T-Cells, 8 hrs., ligation 2	Activated T-Cells	Blood	Cell Line		Uni-ZAP XR
H0163	Human Synovium	Human Synovium	Synovium			Uni-ZAP XR
H0166	Human Prostate Cancer, Stage B2 fraction	Human Prostate Cancer, stage B2	Prostate		disease	Uni-ZAP XR
H0169	Human Prostate Cancer, Stage C fraction	Human Prostate Cancer, stage C	Prostate		disease	Uni-ZAP XR
H0170	12 Week Old Early Stage Human	Twelve Week Old Early Stage Human	Embryo			Uni-ZAP XR
H0171	12 Week Old Early Stage Human, II	Twelve Week Old Early Stage Human	Embryo			Uni-ZAP XR
H0172	Human Fetal Brain, random primed	Human Fetal Brain	Brain			Lambda ZAP II
H0178	Human Fetal Brain	Human Fetal Brain	Brain			Uni-ZAP XR
H0179	Human Neutrophil	Human Neutrophil	Blood	Cell Line		Uni-ZAP XR
H0181	Human Primary Breast	Human Primary Breast	Breast		disease	Uni-ZAP XR

	Cancer	Cancer				
H0188	Human Normal Breast	Human Normal Breast	Breast			Uni-ZAP XR
H0194	Human Cerebellum, subtracted	Human Cerebellum	Brain			pBluescript
H0196	Human Cardiomyopathy, subtracted	Human Cardiomyopathy	Heart			Uni-ZAP XR
H0197	Human Fetal Liver, subtracted	Human Fetal Liver	Liver			Uni-ZAP XR
H0201	Human Hippocampus, subtracted	Human Hippocampus	Brain			pBluescript
H0208	Early Stage Human Lung, subtracted	Human Fetal Lung	Lung			pBluescript
H0212	Human Prostate, subtracted	Human Prostate	Prostate			pBluescript
H0213	Human Pituitary, subtracted	Human Pituitary				Uni-ZAP XR
H0216	Supt cells, cyclohexamide treated, subtracted	Cyclohexamide Treated Cem, Jurkat, Raji, and Supt	Blood	Cell Line		pBluescript
H0220	Activated T-Cells, 4 hrs, subtracted	Activated T-Cells	Blood	Cell Line		Uni-ZAP XR
H0231	Human Colon, subtraction	Human Colon				pBluescript
H0232	Human Colon, differential expression	Human Colon				pBluescript
H0235	Human colon cancer, metastasized to liver, subtraction	Human Colon Cancer, metastasized to liver	Liver			pBluescript
H0239	Human Kidney Tumor	Human Kidney Tumor	Kidney		disease	Uni-ZAP XR
H0241	C7MCF7 cell line, estrogen treated, subtraction	C7MCF7 Cell Line, estrogen treated	Breast	Cell Line		Uni-ZAP XR
H0242	Human Fetal Heart, Differential (Fetal-Specific)	Human Fetal Heart	Heart			pBluescript
H0244	Human 8 Week Whole Embryo, subtracted	Human 8 Week Old Embryo	Embryo			Uni-ZAP XR

H0250	Human Activated Monocytes	Human Monocytes				Uni-ZAP XR
H0251	Human Chondrosarcoma	Human Chondrosarcoma	Cartilage		disease	Uni-ZAP XR
H0252	Human Osteosarcoma	Human Osteosarcoma	Bone		disease	Uni-ZAP XR
H0253	Human adult testis, large inserts	Human Adult Testis	Testis			Uni-ZAP XR
H0254	Breast Lymph node cDNA library	Breast Lymph Node	Lymph Node			Uni-ZAP XR
H0255	breast lymph node CDNA library	Breast Lymph Node	Lymph Node			Lambda ZAP II
H0257	HL-60, PMA 4H	HL-60 Cells, PMA stimulated 4H	Blood	Cell Line		Uni-ZAP XR
H0261	H. cerebellum, Enzyme subtracted	Human Cerebellum	Brain			Uni-ZAP XR
H0263	human colon cancer	Human Colon Cancer	Colon		disease	Lambda ZAP II
H0264	human tonsils	Human Tonsil	Tonsil			Uni-ZAP XR
H0265	Activated T-Cell (12hs)/Thiouridine labelledEco	T-Cells	Blood	Cell Line		Uni-ZAP XR
H0266	Human Microvascular Endothelial Cells, fract. A	HMEC	Vein	Cell Line		Lambda ZAP II
H0268	Human Umbilical Vein Endothelial Cells, fract. A	HUVE Cells	Umbilical vein	Cell Line		Lambda ZAP II
H0270	HPAS (human pancreas, subtracted)	Human Pancreas	Pancreas			Uni-ZAP XR
H0271	Human Neutrophil, Activated	Human Neutrophil - Activated	Blood	Cell Line		Uni-ZAP XR
H0272	HUMAN TONSILS, FRACTION 2	Human Tonsil	Tonsil			Uni-ZAP XR
H0279	K562 cells	K562 Cell line	cell line	Cell Line		ZAP Express
H0280	K562 + PMA (36 hrs)	K562 Cell line	cell line	Cell Line		ZAP Express
H0284	Human OB MG63 control fraction I	Human Osteoblastoma MG63 cell line	Bone	Cell Line		Uni-ZAP XR

H0286	Human OB MG63 treated (10 nM E2) fraction I	Human Osteoblastoma MG63 cell line	Bone	Cell Line		Uni-ZAP XR
H0288	Human OB HOS control fraction I	Human Osteoblastoma HOS cell line	Bone	Cell Line		Uni-ZAP XR
H0292	Human OB HOS treated (10 nM E2) fraction I	Human Osteoblastoma HOS cell line	Bone	Cell Line		Uni-ZAP XR
H0293	W1 38 cells					Uni-ZAP XR
H0294	Amniotic Cells - TNF induced	Amniotic Cells - TNF induced	Placenta	Cell Line		Uni-ZAP XR
H0295	Amniotic Cells - Primary Culture	Amniotic Cells - Primary Culture	Placenta	Cell Line		Uni-ZAP XR
H0305	CD34 positive cells (Cord Blood)	CD34 Positive Cells	Cord Blood			ZAP Express
H0306	CD34 depleted Buffy Coat (Cord Blood)	CD34 Depleted Buffy Coat (Cord Blood)	Cord Blood			ZAP Express
H0309	Human Chronic Synovitis	Synovium, Chronic Synovitis/ Osteoarthritis	Synovium		disease	Uni-ZAP XR
H0310	human caudate nucleus	Brain	Brain			Uni-ZAP XR
H0316	HUMAN STOMACH	Human Stomach	Stomach			Uni-ZAP XR
H0318	HUMAN B CELL LYMPHOMA	Human B Cell Lymphoma	Lymph Node		disease	Uni-ZAP XR
H0327	human corpus colosum	Human Corpus Callosum	Brain			Uni-ZAP XR
H0328	human ovarian cancer	Ovarian Cancer	Ovary		disease	Uni-ZAP XR
H0329	Dermatofibrosarcoma Protuberance	Dermatofibrosarcoma Protuberans	Skin		disease	Uni-ZAP XR
H0331	Hepatocellular Tumor	Hepatocellular Tumor	Liver		disease	Lambda ZAP II
H0333	Hemangiopericytoma	Hemangiopericytoma	Blood vessel		disease	Lambda ZAP II
H0334	Kidney cancer	Kidney Cancer	Kidney		disease	Uni-ZAP XR
H0339	Duodenum	Duodenum				Uni-ZAP XR
H0341	Bone Marrow Cell Line (RS4;11)	Bone Marrow Cell Line RS4;11	Bone Marrow	Cell Line		Uni-ZAP XR
H0345	SKIN	Skin - 4000868H	Skin			Uni-ZAP XR
H0351	Glioblastoma	Glioblastoma	Brain		disease	Uni-ZAP XR

H0352	wilm's tumor	Wilm's Tumor				disease	Uni-ZAP XR
H0354	Human Leukocytes	Human Leukocytes			Cell Line		pCMVSPORT 1
H0355	Human Liver	Human Liver, normal Adult					pCMVSPORT 1
H0356	Human Kidney	Human Kidney		Kidney			pCMVSPORT 1
H0357	H. Normalized Fetal Liver, II	Human Fetal Liver		Liver			Uni-ZAP XR
H0362	HeLa cell line	HELA CELL LINE					pSport1
H0366	L428 cell line	L428					ZAP Express
H0369	H. Atrophic Endometrium	Atrophic Endometrium and myometrium					Uni-ZAP XR
H0370	H. Lymph node breast Cancer	Lymph node with Met. Breast Cancer				disease	Uni-ZAP XR
H0373	Human Heart	Human Adult Heart		Heart			pCMVSPORT 1
H0374	Human Brain	Human Brain					pCMVSPORT 1
H0375	Human Lung	Human Lung					pCMVSPORT 1
H0379	Human Tongue, frac 1	Human Tongue					pSport1
H0380	Human Tongue, frac 2	Human Tongue					pSport1
H0383	Human Prostate BPH, re-excision	Human Prostate BPH					Uni-ZAP XR
H0388	Human Rejected Kidney, 704 re-excision	Human Rejected Kidney				disease	pBluescript
H0390	Human Amygdala Depression, re-excision	Human Amygdala Depression				disease	pBluescript
H0391	H. Meningioma, M6	Human Meningioma		brain			pSport1
H0392	H. Meningioma, M1	Human Meningioma		brain			pSport1
H0393	Fetal Liver, subtraction II	Human Fetal Liver		Liver			pBluescript
H0395	A1-CELL LINE	Redd-Sternberg cell					ZAP Express
H0402	CD34 depleted Buffy Coat (Cord Blood), re-excision	CD34 Depleted Buffy Coat (Cord Blood)		Cord Blood			ZAP Express
H0406	H Amygdala Depression, subtracted	Human Amygdala Depression					Uni-ZAP XR
H0411	H Female Bladder, Adult	Human Female Adult Bladder		Bladder			pSport1

H0412	Human umbilical vein endothelial cells, IL-4 induced	HUVE Cells	Umbilical vein	Cell Line		pSport1
H0413	Human Umbilical Vein Endothelial Cells, uninduced	HUVE Cells	Umbilical vein	Cell Line		pSport1
H0415	H. Ovarian Tumor, II, OV5232	Ovarian Tumor, OV5232	Ovary		disease	pCMVSPORT 2.0
H0416	Human Neutrophils, Activated, re-excision	Human Neutrophil - Activated	Blood	Cell Line		pBluescript
H0417	Human Pituitary, subtraced VIII	Human Pituitary				pBluescript
H0419	Bone Cancer, re-excision	Bone Cancer				Uni-ZAP XR
H0421	Human Bone Marrow, re-excision	Bone Marrow				pBluescript
H0422	T-Cell PHA 16 hrs	T-Cells	Blood	Cell Line		pSport1
H0423	T-Cell PHA 24 hrs	T-Cells	Blood	Cell Line		pSport1
H0424	Human Pituitary, sub IX	Human Pituitary				pBluescript
H0427	Human Adipose	Human Adipose, left hipipoma				pSport1
H0428	Human Ovary	Human Ovary Tumor	Ovary			pSport1
H0429	K562 + PMA (36 hrs), re-excision	K562 Cell line	cell line	Cell Line		ZAP Express
H0431	H. Kidney Medulla, re-excision	Kidney medulla	Kidney			pBluescript
H0433	Human Umbilical Vein Endothelial cells, frac B, re-excision	HUVE Cells	Umbilical vein	Cell Line		pBluescript
H0435	Ovarian Tumor 10-3-95	Ovarian Tumor, OV350721	Ovary			pCMVSPORT 2.0
H0436	Resting T-Cell Library, II	T-Cells	Blood	Cell Line		pSport1
H0437	H Umbilical Vein Endothelial Cells, frac A, re-excision	HUVE Cells	Umbilical vein	Cell Line		Lambda ZAP II

H0438	H. Whole Brain #2, re-excision	Human Whole Brain #2				ZAP Express
H0439	Human Eosinophils	Eosinophils				pBluescript
H0441	H. Kidney Cortex, subtracted	Kidney cortex	Kidney			pBluescript
H0443	H. Adipose, subtracted	Human Adipose, left hip lipoma				pSport1
H0444	Spleen metastatic melanoma	Spleen, Metastatic malignant melanoma	Spleen		disease	pSport1
H0445	Spleen, Chronic lymphocytic leukemia	Human Spleen, CLL	Spleen		disease	pSport1
H0450	CD34+cells, II	CD34 positive cells				pCMV Sport 2.0
H0455	H. Striatum Depression, subt	Human Brain, Striatum Depression	Brain			pBluescript
H0457	Human Eosinophils	Human Eosinophils				pSport1
H0477	Human Tonsil, Lib 3	Human Tonsil	Tonsil			pSport1
H0478	Salivary Gland, Lib 2	Human Salivary Gland	Salivary gland			pSport1
H0479	Salivary Gland, Lib 3	Human Salivary Gland	Salivary gland			pSport1
H0483	Breast Cancer cell line, MDA 36	Breast Cancer Cell line, MDA 36				pSport1
H0484	Breast Cancer Cell line, angiogenic	Breast Cancer Cell line, Angiogenic, 36T3				pSport1
H0486	Hodgkin's Lymphoma II	Hodgkin's Lymphoma II			disease	pCMV Sport 2.0
H0487	Human Tonsils, lib I	Human Tonsils				pCMV Sport 2.0
H0488	Human Tonsils, Lib 2	Human Tonsils				pCMV Sport 2.0
H0492	HL-60, RA 4h, Subtracted	HL-60 Cells, RA stimulated for 4H	Blood	Cell Line		Uni-ZAP XR
H0494	Keratinocyte	Keratinocyte				pCMV Sport 2.0
H0497	HEL cell line	HEL cell line		HEL 92.1.7		pSport1
H0505	Human Astrocyte	Human Astrocyte				pSport1
H0506	Ulcerative Colitis	Colon	Colon			pSport1
H0509	Liver, Hepatoma	Human Liver, Hepatoma, patient 8	Liver		disease	pCMV Sport 3.0

H0510	Human Liver, normal	Human Liver, normal, Patient # 8	Liver			pCMVSPORT 3.0
H0518	pBMC stimulated w/ poly I/C	pBMC stimulated with poly I/C				pCMVSPORT 3.0
H0519	NTERA2, control	NTERA2, Teratocarcinoma cell line				pCMVSPORT 3.0
H0520	NTERA2 + retinoic acid, 14 days	NTERA2, Teratocarcinoma cell line				pSport1
H0521	Primary Dendritic Cells, lib 1	Primary Dendritic cells				pCMVSPORT 3.0
H0522	Primary Dendritic cells, frac 2	Primary Dendritic cells				pCMVSPORT 3.0
H0525	PCR, pBMC I/C treated	pBMC stimulated with poly I/C				PCR II
H0528	Poly[I]/Poly[C] Normal Lung Fibroblasts	Poly[I]/Poly[C] Normal Lung Fibroblasts				pCMVSPORT 3.0
H0529	Myoloid Progenitor Cell Line	TF-1 Cell Line; Myoloid progenitor cell line				pCMVSPORT 3.0
H0538	Merkel Cells	Merkel cells	Lymph node			pSport1
H0539	Pancreas Islet Cell Tumor	Pancreas Islet Cell Tumour	Pancreas		disease	pSport1
H0542	T Cell helper I	Helper T cell				pCMVSPORT 3.0
H0543	T cell helper II	Helper T cell				pCMVSPORT 3.0
H0544	Human endometrial stromal cells	Human endometrial stromal cells				pCMVSPORT 3.0
H0545	Human endometrial stromal cells-treated with progesterone	Human endometrial stromal cells-treated with proge				pCMVSPORT 3.0
H0546	Human endometrial stromal cells-treated with estradiol	Human endometrial stromal cells-treated with estro				pCMVSPORT 3.0
H0547	NTERA2 teratocarcinoma cell line+retinoic acid (14 days)	NTERA2, Teratocarcinoma cell line				pSport1

H0549	H. Epididymus, caput & corpus	Human Epididymus, caput and corpus					Uni-ZAP XR
H0550	H. Epididymus, cauda	Human Epididymus, cauda					Uni-ZAP XR
H0551	Human Thymus Stromal Cells	Human Thymus Stromal Cells					pCMVSPORT 3.0
H0553	Human Placenta	Human Placenta					pCMVSPORT 3.0
H0555	Rejected Kidney, lib 4	Human Rejected Kidney	Kidney			disease	pCMVSPORT 3.0
H0556	Activated T-cell(12h)/Thiouridine-re-excision	T-Cells	Blood				Uni-ZAP XR
H0559	HL-60, PMA 4H, re-excision	HL-60 Cells, PMA stimulated 4H	Blood				Uni-ZAP XR
H0560	KMH2	KMH2					pCMVSPORT 3.0
H0561	L428	L428					pCMVSPORT 3.0
H0563	Human Fetal Brain, normalized 50021F	Human Fetal Brain					pCMVSPORT 2.0
H0564	Human Fetal Brain, normalized C5001F	Human Fetal Brain					pCMVSPORT 2.0
H0566	Human Fetal Brain, normalized c50F	Human Fetal Brain					pCMVSPORT 2.0
H0569	Human Fetal Brain, normalized CO	Human Fetal Brain					pCMVSPORT 2.0
H0570	Human Fetal Brain, normalized C500H	Human Fetal Brain					pCMVSPORT 2.0
H0571	Human Fetal Brain, normalized C500HE	Human Fetal Brain					pCMVSPORT 2.0
H0572	Human Fetal Brain, normalized AC5002	Human Fetal Brain					pCMVSPORT 2.0
H0574	Hepatocellular Tumor; re-excision	Hepatocellular Tumor	Liver			disease	Lambda ZAP II
H0575	Human Adult Pulmonary;re-excision	Human Adult Pulmonary	Lung				Uni-ZAP XR
H0576	Resting T-Cell; re-excision	T-Cells	Blood				Lambda ZAP II

H0580	Dendritic cells, pooled	Pooled dendritic cells				pCMVSPORT 3.0
H0581	Human Bone Marrow, treated	Human Bone Marrow	Bone Marrow			pCMVSPORT 3.0
H0583	B Cell lymphoma	B Cell Lymphoma	B Cell		disease	pCMVSPORT 3.0
H0585	Activated T-Cells, 12 hrs, re-excision	Activated T-Cells	Blood	Cell Line		Uni-ZAP XR
H0586	Healing groin wound, 6.5 hours post incision	healing groin wound, 6.5 hours post incision - 2/	groin		disease	pCMVSPORT 3.0
H0587	Healing groin wound; 7.5 hours post incision	Groin-2/19/97	groin		disease	pCMVSPORT 3.0
H0589	CD34 positive cells (cord blood), re-ex	CD34 Positive Cells	Cord Blood			ZAP Express
H0590	Human adult small intestine, re-excision	Human Adult Small Intestine	Small Int.			Uni-ZAP XR
H0591	Human T-cell lymphoma; re-excision	T-Cell Lymphoma	T-Cell		disease	Uni-ZAP XR
H0592	Healing groin wound - zero hr post-incision (control)	HGS wound healing project; abdomen			disease	pCMVSPORT 3.0
H0593	Olfactory epithelium; nasal cavity	Olfactory epithelium from roof of left nasal cavity				pCMVSPORT 3.0
H0594	Human Lung Cancer; re-excision	Human Lung Cancer	Lung		disease	Lambda ZAP II
H0595	Stomach cancer (human); re-excision	Stomach Cancer - 5383A (human)			disease	Uni-ZAP XR
H0596	Human Colon Cancer; re-excision	Human Colon Cancer	Colon			Lambda ZAP II
H0597	Human Colon; re-excision	Human Colon				Lambda ZAP II
H0598	Human Stomach; re-excision	Human Stomach	Stomach			Uni-ZAP XR
H0599	Human Adult Heart; re-excision	Human Adult Heart	Heart			Uni-ZAP XR
H0600	Healing Abdomen wound; 70&90 min post	Abdomen			disease	pCMVSPORT 3.0

	incision		Abdomen				disease	
H0602	Healing Abdomen Wound; 21 & 29 days post incision							pCMV Sport 3.0
H0604	Human Pituitary, re-excision		Human Pituitary					pBluescript
H0606	Human Primary Breast Cancer; re-excision		Human Primary Breast Cancer		Breast		disease	Uni-ZAP XR
H0609	H. Leukocytes, normalized cot > 500A		H. Leukocytes					pCMV Sport 1
H0615	Human Ovarian Cancer Reexcision		Ovarian Cancer		Ovary		disease	Uni-ZAP XR
H0616	Human Testes, Reexcision		Human Testes		Testis			Uni-ZAP XR
H0617	Human Primary Breast Cancer Reexcision		Human Primary Breast Cancer		Breast		disease	Uni-ZAP XR
H0618	Human Adult Testes, Large Inserts, Reexcision		Human Adult Testis		Testis			Uni-ZAP XR
H0619	Fetal Heart		Human Fetal Heart		Heart			Uni-ZAP XR
H0620	Human Fetal Kidney; Reexcision		Human Fetal Kidney		Kidney			Uni-ZAP XR
H0622	Human Pancreas Tumor; Reexcision		Human Pancreas Tumor		Pancreas		disease	Uni-ZAP XR
H0623	Human Umbilical Vein; Reexcision		Human Umbilical Vein Endothelial Cells		Umbilical vein			Uni-ZAP XR
H0624	12 Week Early Stage Human II; Reexcision		Twelve Week Old Early Stage Human		Embryo			Uni-ZAP XR
H0625	Ku 812F Basophils Line		Ku 812F Basophils					pSport1
H0627	Saos2 Cells; Vitamin D3 Treated		Saos2 Cell Line; Vitamin D3 Treated					pSport1
H0628	Human Pre-Differentiated Adipocytes		Human Pre-Differentiated Adipocytes					Uni-ZAP XR
H0631	Saos2, Dexamethosone Treated		Saos2 Cell Line; Dexamethosone Treated					pSport1

H0632	Hepatocellular Tumor; re-excision	Hepatocellular Tumor	Liver				Lambda ZAP II
H0633	Lung Carcinoma A549 TNFalpha activated	TNFalpha activated A549-- Lung Carcinoma				disease	pSport1
H0634	Human Testes Tumor, re-excision	Human Testes Tumor	Testis			disease	Uni-ZAP XR
H0635	Human Activated T-Cells, re-excision	Activated T-Cells	Blood		Cell Line		Uni-ZAP XR
H0637	Dendritic Cells From CD34 Cells	Dendritic cells from CD34 cells					pSport1
H0638	CD40 activated monocyte dendritic cells	CD40 activated monocyte dendritic cells					pSport1
H0640	Ficoll Human Stromal Cells, Untreated	Ficoll Human Stromal Cells, Untreated					Other
H0641	LPS activated derived dendritic cells	LPS activated monocyte derived dendritic cells					pSport1
H0642	Hep G2 Cells, lambda library	Hep G2 Cells					Other
H0643	Hep G2 Cells, PCR library	Hep G2 Cells					Other
H0644	Human Placenta (re-excision)	Human Placenta	Placenta				Uni-ZAP XR
H0645	Fetal Heart, re-excision	Human Fetal Heart	Heart				Uni-ZAP XR
H0646	Lung, Cancer (4005313 A3): Invasive Poorly Differentiated Lung Adenocarcinoma,	Metastatic squamous cell lung carcinoma, poorly di					pSport1
H0647	Lung, Cancer (4005163 B7): Invasive, Poorly Diff. Adenocarcinoma, Metastatic	Invasive poorly differentiated lung adenocarcinoma				disease	pSport1
H0648	Ovary, Cancer: (4004562 B6) Papillary Serous Cystic Neoplasm, Low	Papillary Cstic neoplasm of low malignant potentia				disease	pSport1

	Malignant Pot						
H0649	Lung, Normal: (4005313 B1)	Normal Lung					pSport1
H0650	B-Cells	B-Cells					pCMVSPORT 3.0
H0651	Ovary, Normal: (9805C040R)	Normal Ovary					pSport1
H0652	Lung, Normal: (4005313 B1)	Normal Lung					pSport1
H0653	Stromal Cells	Stromal Cells					pSport1
H0656	B-cells (unstimulated)	B-cells (unstimulated)					pSport1
H0657	B-cells (stimulated)	B-cells (stimulated)					pSport1
H0658	Ovary, Cancer (9809C332): Poorly differentiated adenocarcinoma	9809C332- Poorly differentiate	Ovary & Fallopian Tubes			disease	pSport1
H0659	Ovary, Cancer (15395A1F): Grade II Papillary Carcinoma	Grade II Papillary Carcinoma, Ovary	Ovary			disease	pSport1
H0660	Ovary, Cancer: (15799A1F) Poorly differentiated carcinoma	Poorly differentiated carcinoma, ovary				disease	pSport1
H0661	Breast, Cancer: (4004943 A5)	Breast cancer				disease	pSport1
H0662	Breast, Normal: (4005522B2)	Normal Breast - #4005522(B2)	Breast				pSport1
H0663	Breast, Cancer: (4005522 A2)	Breast Cancer - #4005522(A2)	Breast			disease	pSport1
H0664	Breast, Cancer: (9806C012R)	Breast Cancer	Breast			disease	pSport1
H0665	Stromal cells 3.88	Stromal cells 3.88					pSport1
H0666	Ovary, Cancer: (4004332 A2)	Ovarian Cancer, Sample #4004332A2				disease	pSport1
H0667	Stromal cells(HBM3.18)	Stromal cell(HBM 3.18)					pSport1

H0668	stromal cell clone 2.5	stromal cell clone 2.5					pSport1
H0670	Ovary, Cancer(4004650 A3): Well-Differentiated Micropapillary Serous Carcinoma	Ovarian Cancer - 4004650A3					pSport1
H0671	Breast, Cancer: (9802C02OE)	Breast Cancer- Sample # 9802C02OE					pSport1
H0672	Ovary, Cancer: (4004576 A8)	Ovarian Cancer(4004576A8)	Ovary				pSport1
H0673	Human Prostate Cancer, Stage B2; re-excision	Human Prostate Cancer, stage B2	Prostate				Uni-ZAP XR
H0674	Human Prostate Cancer, Stage C; re-excision	Human Prostate Cancer, stage C	Prostate				Uni-ZAP XR
H0675	Colon, Cancer: (9808C064R)	Colon Cancer 9808C064R					pCMVSPORT 3.0
H0676	Colon, Cancer: (9808C064R)-total RNA	Colon Cancer 9808C064R					pCMVSPORT 3.0
H0677	TNFR degenerate oligo	B-Cells					PCR II
H0682	Serous Papillary Adenocarcinoma	serous papillary adenocarcinoma (9606G304SPA3B)					pCMVSPORT 3.0
H0683	Ovarian Serous Papillary Adenocarcinoma	Serous papillary adenocarcinoma, stage 3C (9804G01)					pCMVSPORT 3.0
H0684	Serous Papillary Adenocarcinoma	Ovarian Cancer-9810G606	Ovaries				pCMVSPORT 3.0
H0685	Adenocarcinoma of Ovary, Human Cell Line, # OVCAR-3	Adenocarcinoma of Ovary, Human Cell Line, # OVCAR-3					pCMVSPORT 3.0
H0686	Adenocarcinoma of Ovary, Human Cell Line	Adenocarcinoma of Ovary, Human Cell Line, # SW-626					pCMVSPORT 3.0
H0687	Human normal ovary(#9610G215)	Human normal ovary(#9610G215)	Ovary				pCMVSPORT 3.0

H0688	Human Ovarian Cancer(#9807G017)	Human Ovarian cancer(#9807G017),mRNA from Maura Ru					pCMVSPORT 3.0
H0689	Ovarian Cancer	Ovarian Cancer, #9806G019					pCMVSPORT 3.0
H0692	BLyS Receptor from Expression Cloning	B Cell Lymphoma	B Cell				pCMVSPORT 3.0
H0693	Normal Prostate #ODQ3958EN	Normal Prostate Tissue # ODQ3958EN					pCMVSPORT 3.0
H0694	Prostate gland adenocarcinoma	Prostate gland, adenocarcinoma, mod/diff, gleason	prostate gland				pCMVSPORT 3.0
N0006	Human Fetal Brain	Human Fetal Brain					
S0001	Brain frontal cortex	Brain frontal cortex	Brain				Lambda ZAP II
S0002	Monocyte activated	Monocyte-activated	blood	Cell Line			Uni-ZAP XR
S0003	Human Osteoclastoma	Osteoclastoma	bone		disease		Uni-ZAP XR
S0004	Prostate	Prostate BPH	Prostate				Lambda ZAP II
S0006	Neuroblastoma	Human Neural Blastoma			disease		pCDNA
S0007	Early Stage Human Brain	Human Fetal Brain					Uni-ZAP XR
S0010	Human Amygdala	Amygdala					Uni-ZAP XR
S0011	STROMAL - OSTEOCLASTOMA	Osteoclastoma	bone		disease		Uni-ZAP XR
S0013	Prostate	Prostate	prostate				Uni-ZAP XR
S0016	Kidney Pyramids	Kidney pyramids	Kidney				Uni-ZAP XR
S0022	Human Osteoclastoma Stromal Cells - unamplified	Osteoclastoma Stromal Cells					Uni-ZAP XR
S0024	Human Kidney Medulla - unamplified	Human Kidney Medulla					
S0026	Stromal cell TF274	stromal cell	Bone marrow	Cell Line			Uni-ZAP XR
S0027	Smooth muscle, serum treated	Smooth muscle	Pulmonary artery	Cell Line			Uni-ZAP XR
S0028	Smooth muscle,control	Smooth muscle	Pulmonary artery	Cell Line			Uni-ZAP XR

S0029	brain stem	Brain stem	brain			Uni-ZAP XR
S0031	Spinal cord	Spinal cord	spinal cord			Uni-ZAP XR
S0032	Smooth muscle-IL1b induced	Smooth muscle	Pulmonary artery		Cell Line	Uni-ZAP XR
S0036	Human Substantia Nigra	Human Substantia Nigra				Uni-ZAP XR
S0037	Smooth muscle, IL1b induced	Smooth muscle	Pulmonary artery		Cell Line	Uni-ZAP XR
S0038	Human Whole Brain #2 - Oligo dT > 1.5Kb	Human Whole Brain #2				ZAP Express
S0039	Hypothalamus	Hypothalamus	Brain			Uni-ZAP XR
S0040	Adipocytes	Human Adipocytes from Osteoclastoma				Uni-ZAP XR
S0042	Testes	Human Testes				ZAP Express
S0044	Prostate BPH	prostate BPH	Prostate	disease		Uni-ZAP XR
S0045	Endothelial cells-control	Endothelial cell	endothelial cell-lung		Cell Line	Uni-ZAP XR
S0046	Endothelial-induced	Endothelial cell	endothelial cell-lung		Cell Line	Uni-ZAP XR
S0049	Human Brain, Striatum	Human Brain, Striatum				Uni-ZAP XR
S0050	Human Frontal Cortex, Schizophrenia	Human Frontal Cortex, Schizophrenia		disease		Uni-ZAP XR
S0051	Human Hypothalamus, Schizophrenia ^a	Human Hypothalamus, Schizophrenia		disease		Uni-ZAP XR
S0052	neutrophils control	human neutrophils	blood		Cell Line	Uni-ZAP XR
S0053	Neutrophils IL-1 and LPS induced	human neutrophil induced	blood		Cell Line	Uni-ZAP XR
S0106	STRIATUM DEPRESSION		BRAIN	disease		Uni-ZAP XR
S0110	Brain Amygdala Depression		Brain	disease		Uni-ZAP XR
S0112	Hypothalamus		Brain			Uni-ZAP XR
S0114	Anergic T-cell	Anergic T-cell			Cell Line	Uni-ZAP XR

S0116	Bone marrow	Bone marrow	Bone marrow				Uni-ZAP XR
S0122	Osteoclastoma-normalized A	Osteoclastoma	bone			disease	pBluescript
S0124	Smooth muscle-edited A	Smooth muscle	Pulmonary artery		Cell Line		Uni-ZAP XR
S0126	Osteoblasts	Osteoblasts	Knee		Cell Line		Uni-ZAP XR
S0132	Epithelial-TNF α and INF induced	Airway Epithelial					Uni-ZAP XR
S0134	Apoptotic T-cell	apoptotic cells			Cell Line		Uni-ZAP XR
S0136	PERM TF274	stromal cell	Bone marrow		Cell Line		Lambda ZAP II
S0140	eosinophil-IL5 induced	eosinophil	lung		Cell Line		Uni-ZAP XR
S0142	Macrophage-oxLDL	macrophage-oxidized LDL treated	blood		Cell Line		Uni-ZAP XR
S0144	Macrophage (GM-CSF treated)	Macrophage (GM-CSF treated)					Uni-ZAP XR
S0148	Normal Prostate	Prostate	prostate				Uni-ZAP XR
S0150	LNCAP prostate cell line	LNCAP Cell Line	Prostate		Cell Line		Uni-ZAP XR
S0152	PC3 Prostate cell line	PC3 prostate cell line					Uni-ZAP XR
S0176	Prostate, normal, subtraction I	Prostate	prostate				Uni-ZAP XR
S0182	Human B Cell 8866	Human B- Cell 8866					Uni-ZAP XR
S0192	Synovial Fibroblasts (control)	Synovial Fibroblasts					pSport1
S0194	Synovial hypoxia	Synovial Fibroblasts					pSport1
S0196	Synovial IL-1/TNF stimulated	Synovial Fibroblasts					pSport1
S0206	Smooth Muscle- HASTE normalized	Smooth muscle	Pulmonary artery		Cell Line		pBluescript
S0208	Mesangial cell, frac 1	Mesangial cell					pSport1
S0210	Mesangial cell, frac 2	Mesangial cell					pSport1
S0212	Bone Marrow Stromal Cell, untreated	Bone Marrow Stromal Cell, untreated					pSport1
S0214	Human Osteoclastoma, re-	Osteoclastoma	bone			disease	Uni-ZAP XR

	excision	human neutrophil induced	blood	Cell Line		
S0216	Neutrophils IL-1 and LPS induced	human neutrophil induced	blood	Cell Line		Uni-ZAP XR
S0218	Apoptotic T-cell, re-excision	apoptotic cells		Cell Line		Uni-ZAP XR
S0220	H. hypothalamus, frac A, re-excision	Hypothalamus	Brain			ZAP Express
S0222	H. Frontal cortex, epileptic; re-excision	H. Brain, Frontal Cortex, Epileptic	Brain		disease	Uni-ZAP XR
S0242	Synovial Fibroblasts (III/TNF), subt	Synovial Fibroblasts				pSport1
S0250	Human Osteoblasts II	Human Osteoblasts	Femur		disease	pCMV Sport 2.0
S0260	Spinal Cord, re-excision	Spinal cord	spinal cord			Uni-ZAP XR
S0276	Synovial hypoxia-RSF subtracted	Synovial fobroblasts (rheumatoid)	Synovial tissue			pSport1
S0278	H Macrophage (GM-CSF treated), re-excision	Macrophage (GM-CSF treated)				Uni-ZAP XR
S0280	Human Adipose Tissue, re-excision	Human Adipose Tissue				Uni-ZAP XR
S0282	Brain Frontal Cortex, re-excision	Brain frontal cortex	Brain			Lambda ZAP II
S0294	Larynx tumor	Larynx tumor	Larynx, vocal cord		disease	pSport1
S0296	Normal lung	Normal lung	Lung			pSport1
S0298	Bone marrow stroma, treated	Bone marrow stroma, treated SB	Bone marrow			pSport1
S0300	Frontal lobe, dementia; re-excision	Frontal Lobe dementia/Alzheimer's	Brain			Uni-ZAP XR
S0308	Spleen/normal	Spleen normal				pSport1
S0310	Normal trachea	Normal trachea				pSport1
S0312	Human osteoarthritis; fraction II	Human osteoarthritis cartilage			disease	pSport1
S0314	Human	Human osteoarthritis			disease	pSport1

	osteoarthritis;fraction I	cartilage				
S0316	Human Normal Cartilage,Fraction I	Human Normal Cartilage				pSportI
S0318	Human Normal Cartilage Fraction II	Human Normal Cartilage				pSportI
S0328	Palate carcinoma	Palate carcinoma	Uvula		disease	pSportI
S0330	Palate normal	Palate normal	Uvula			pSportI
S0334	Human Normal Cartilage Fraction III	Human Normal Cartilage				pSportI
S0338	Human Osteoarthritic Cartilage Fraction III	Human osteoarthritic cartilage			disease	pSportI
S0340	Human Osteoarthritic Cartilage Fraction IV	Human osteoarthritic cartilage			disease	pSportI
S0342	Adipocytes;re-excision	Human Adipocytes from Osteoclastoma				Uni-ZAP XR
S0344	Macrophage-oxLDL; re-excision	macrophage-oxidized LDL treated	blood	Cell Line		Uni-ZAP XR
S0346	Human Amygdala;re-excision	Amygdala				Uni-ZAP XR
S0352	Larynx Carcinoma	Larynx carcinoma			disease	pSportI
S0354	Colon Normal II	Colon Normal	Colon			pSportI
S0356	Colon Carcinoma	Colon Carcinoma	Colon		disease	pSportI
S0358	Colon Normal III	Colon Normal	Colon			pSportI
S0360	Colon Tumor II	Colon Tumor	Colon		disease	pSportI
S0362	Human Gastrocnemius	Gastrocnemius muscle				pSportI
S0364	Human Quadriceps	Quadriceps muscle				pSportI
S0366	Human Soleus	Soleus Muscle				pSportI
S0370	Larynx carcinoma II	Larynx carcinoma			disease	pSportI
S0372	Larynx carcinoma III	Larynx carcinoma			disease	pSportI
S0374	Normal colon	Normal colon				pSportI
S0376	Colon Tumor	Colon Tumor			disease	pSportI
S0378	Pancreas normal PCA4 No	Pancreas Normal PCA4 No				pSportI

S0380	Pancreas Tumor PCA4 Tu	Pancreas Tumor PCA4 Tu				disease	pSport1
S0386	Human Whole Brain, re-excision	Whole brain	Brain				ZAP Express
S0388	Human Hypothalamus, schizophrenia, re-excision	Human Hypothalamus, Schizophrenia				disease	Uni-ZAP XR
S0390	Smooth muscle, control; re-excision	Smooth muscle	Pulmonary artery	Cell Line			Uni-ZAP XR
S0392	Salivary Gland	Salivary gland; normal					pSport1
S0398	Testis; normal	Testis; normal					pSport1
S0400	Brain; normal	Brain; normal					pSport1
S0404	Rectum normal	Rectum, normal					pSport1
S0406	Rectum tumour	Rectum tumour					pSport1
S0408	Colon, normal	Colon, normal					pSport1
S0410	Colon, tumour	Colon, tumour					pSport1
S0412	Temporal cortex-Alzheimer; subtraced	Temporal cortex, alzheimer				disease	Other
S0414	Hippocampus, Alzheimer Subtraced	Hippocampus, Alzheimer Subtraced					Other
S0418	CHME Cell Line;treated 5 hrs	CHME Cell Line; treated					pCMVSPORT 3.0
S0420	CHME Cell Line,untreated	CHME Cell line, untreated					pSport1
S0422	Mo7e Cell Line GM-CSF treated (1ng/ml)	Mo7e Cell Line GM-CSF treated (1ng/ml)					pCMVSPORT 3.0
S0424	TF-1 Cell Line GM-CSF Treated	TF-1 Cell Line GM-CSF Treated					pSport1
S0426	Monocyte activated; re-excision	Monocyte-activated	blood	Cell Line			Uni-ZAP XR
S0428	Neutrophils control; re-excision	human neutrophils	blood	Cell Line			Uni-ZAP XR
S0430	Aryepiglottitis Normal	Aryepiglottitis Normal					pSport1
S0432	Sinus piniformis Tumour	Sinus piniformis Tumour					pSport1
S0434	Stomach Normal	Stomach Normal				disease	pSport1

S0436	Stomach Tumour	Stomach Tumour						pSport1	
S0438	Liver Normal Met5No	Liver Normal Met5No						pSport1	
S0440	Liver Tumour Met 5 Tu	Liver Tumour						pSport1	
S0442	Colon Normal	Colon Normal						pSport1	
S0444	Colon Tumor	Colon Tumour					disease	pSport1	
S0446	Tongue Tumour	Tongue Tumour						pSport1	
S0448	Larynx Normal	Larynx Normal						pSport1	
S0450	Larynx Tumour	Larynx Tumour						pSport1	
S0452	Thymus	Thymus						pSport1	
S0458	Thyroid Normal (SDCA2 No)	Thyroid normal						pSport1	
S0460	Thyroid Tumour	Thyroid Tumour						pSport1	
S0462	Thyroid Thyroiditis	Thyroid Thyroiditis						pSport1	
S0464	Larynx Normal	Larynx Normal						pSport1	
S0468	Ea.hy.926 cell line	Ea.hy.926 cell line						pSport1	
S0474	Human blood platelets	Platelets			Blood platelets			Other	
S3012	Smooth Muscle Serum Treated, Norm	Smooth muscle			Pulmonary artery		Cell Line	pBluescript	
S3014	Smooth muscle, serum induced, re-exc	Smooth muscle			Pulmonary artery		Cell Line	pBluescript	
S6014	H. hypothalamus, frac A	Hypothalamus			Brain			ZAP Express	
S6016	H. Frontal Cortex, Epileptic	H. Brain, Frontal Cortex, Epileptic			Brain			Uni-ZAP XR	
S6022	H. Adipose Tissue	Human Adipose Tissue						Uni-ZAP XR	
S6024	Alzheimers, spongy change	Alzheimer's/Spongy change			Brain			Uni-ZAP XR	
S6026	Frontal Lobe, Dementia	Frontal Lobe dementia/Alzheimer's			Brain			Uni-ZAP XR	
S6028	Human Manic Depression Tissue	Human Manic depression tissue			Brain			Uni-ZAP XR	
T0001	Human Brown Fat	Brown Fat						pBluescript SK-	
T0002	Activated T-cells	Activated T-Cell, PBL fraction			Blood		Cell Line	pBluescript SK-	

T0003	Human Fetal Lung	Human Fetal Lung					pBluescript SK-
T0004	Human White Fat	Human White Fat					pBluescript SK-
T0006	Human Pineal Gland	Human Pineal Gland					pBluescript SK-
T0010	Human Infant Brain	Human Infant Brain					Other
T0023	Human Pancreatic Carcinoma	Human Pancreatic Carcinoma				disease	pBluescript SK-
T0039	HSA 172 Cells	Human HSA 172 cell line					pBluescript SK-
T0040	HSC172 cells	SA172 Cells					pBluescript SK-
T0041	Jurkat T-cell G1 phase	Jurkat T-cell					pBluescript SK-
T0042	Jurkat T-Cell, S phase	Jurkat T-Cell Line					pBluescript SK-
T0048	Human Aortic Endothelium	Human Aortic Endothelium					pBluescript SK-
T0049	Aorta endothelial cells + TNF- α	Aorta endothelial cells					pBluescript SK-
T0060	Human White Adipose	Human White Fat					pBluescript SK-
T0067	Human Thyroid	Human Thyroid					pBluescript SK-
T0068	Normal Ovary, Premenopausal	Normal Ovary, Premenopausal					pBluescript SK-
T0069	Human Uterus, normal	Human Uterus, normal					pBluescript SK-
T0070	Human Adrenal Gland	Human Adrenal Gland					pBluescript SK-
T0071	Human Bone Marrow	Human Bone Marrow					pBluescript SK-
T0082	Human Adult Retina	Human Adult Retina					pBluescript SK-
T0104	HCC cell line metastasis to liver						pBluescript SK-
T0109	Human (HCC) cell line liver (mouse) metastasis, remake						pBluescript SK-
T0110	Human colon carcinoma (HCC) cell line, remake						pBluescript SK-
T0114	Human (Caco-2) cell line, adenocarcinoma, colon, remake						pBluescript SK-
T0115	Human Colon Carcinoma						pBluescript SK-

	(HCC) cell line								
L0002	Atrium cDNA library Human heart								
L0005	Clontech human aorta polyA+ mRNA (#6572)								
L0009	EST from 8p21.3-p22								
L0021	Human adult (K.Okubo)								
L0022	Human adult lung 3" directed Mbol cDNA								
L0032	Human chromosome 12p cDNAs								
L0040	Human colon mucosa								
L0041	Human epidermal keratinocyte								
L0055	Human promyelocyte								
L0065	Liver HepG2 cell line.								
L0105	Human aorta polyA+ (TFujiwara)	aorta							
L0109	Human brain cDNA	brain							
L0142	Human placenta cDNA (TFujiwara)	placenta							
L0143	Human placenta polyA+ (TFujiwara)	placenta							
L0157	Human fetal brain (TFujiwara)		brain						
L0163	Human heart cDNA (YNakamura)		heart						
L0351	Infant brain, Bento Soares								BA, M13-derived
L0352	Normalized infant brain, Bento Soares								BA, M13-derived
L0361	Stratagene ovary (#937217)		ovary						Bluescript SK
L0362	Stratagene ovarian cancer								Bluescript SK-

	(#937219)							
L0363	NCI CGAP GC2		germ cell tumor					Bluescript SK-
L0364	NCI CGAP GC5		germ cell tumor					Bluescript SK-
L0366	Stratagene schizo brain S11		schizophrenic brain S-11 frontal lobe					Bluescript SK-
L0369	NCI CGAP AA1		adrenal adenoma		adrenal gland			Bluescript SK-
L0371	NCI CGAP Br3		breast tumor		breast			Bluescript SK-
L0372	NCI CGAP Co12		colon tumor		colon			Bluescript SK-
L0373	NCI CGAP Co11		tumor		colon			Bluescript SK-
L0374	NCI CGAP Co2		tumor		colon			Bluescript SK-
L0375	NCI CGAP Kid6		kidney tumor		kidney			Bluescript SK-
L0376	NCI CGAP Lar1		larynx		larynx			Bluescript SK-
L0378	NCI CGAP Lu1		lung tumor		lung			Bluescript SK-
L0381	NCI CGAP HN4		squamous cell carcinoma		pharynx			Bluescript SK-
L0382	NCI CGAP Pr25		epithelium (cell line)		prostate			Bluescript SK-
L0383	NCI CGAP Pr24		invasive tumor (cell line)		prostate			Bluescript SK-
L0384	NCI CGAP Pr23		prostate tumor		prostate			Bluescript SK-
L0387	NCI CGAP GCB0		germinal center B-cells		tonsil			Bluescript SK-
L0388	NCI CGAP HN6		normal gingiva (cell line from immortalized kerati					Bluescript SK-
L0411	1-NIB							Lafmid BA
L0414	b4HB3MA							Lafmid BA
L0422	b4HB3MA-Cot12-HAP-B							Lafmid BA
L0435	Infant brain, LNL array of Dr. M. Soares 1NIB							lafmid BA
L0438	normalized infant brain cDNA		total brain		brain			lafmid BA
L0439	Soares infant brain 1NIB				whole brain			Lafmid BA
L0442	4HB3MK							Lafmid BK
L0455	Human retina cDNA randomly primed sublibrary		retina		eye			lambda gt10

L0456	Human retina cDNA Tsp509I-cleaved sublibrary	retina	eye			lambda gt10
L0457	multi-tissue normalized short-fragment	multi-tissue	pooled			lambda gt10
L0468	HE6W					lambda zap
L0471	Human fetal heart, Lambda ZAP Express					Lambda ZAP Express
L0475	KG1-a Lambda Zap Express cDNA library			KG1-a		Lambda Zap Express (Stratagene)
L0481	CD34+DIRECTIONAL					Lambda ZAPII
L0483	Human pancreatic islet					Lambda ZAPII
L0485	STRATAGENE Human skeletal muscle cDNA library, cat. #936215.	skeletal muscle	leg muscle			Lambda ZAPII
L0493	NCI CGAP_Ov26	papillary serous carcinoma	ovary			pAMP1
L0497	NCI CGAP_HSC4	CD34+, CD38- from normal bone marrow donor	bone marrow			pAMP1
L0498	NCI CGAP_HSC3	CD34+, T negative, patient with chronic myelogenous	bone marrow			pAMP1
L0500	NCI CGAP_Brm20	oligodendroglioma	brain			pAMP1
L0504	NCI CGAP_Br13	breast carcinoma in situ	breast			pAMP1
L0512	NCI CGAP_Ov36	borderline ovarian carcinoma	ovary			pAMP1
L0514	NCI CGAP_Ov31	papillary serous carcinoma	ovary			pAMP1
L0515	NCI CGAP_Ov32	papillary serous carcinoma	ovary			pAMP1
L0517	NCI CGAP_Pr1					pAMP10
L0518	NCI CGAP_Pr2					pAMP10
L0519	NCI CGAP_Pr3					pAMP10
L0520	NCI CGAP_Alv1	alveolar rhabdomyosarcoma				pAMP10
L0521	NCI CGAP_Ew1	Ewing's sarcoma				pAMP10
L0523	NCI CGAP_Lip2	liposarcoma				pAMP10
L0526	NCI CGAP_Pr12	metastatic prostate bone lesion				pAMP10
L0527	NCI CGAP_Ov2	ovary				pAMP10

L0528	NCI CGAP_Pr5	prostate				pAMP10
L0529	NCI CGAP_Pr6	prostate				pAMP10
L0530	NCI CGAP_Pr8	prostate				pAMP10
L0531	NCI CGAP_Pr20	prostate metastasis, liver				pAMP10
L0533	NCI CGAP_HSC1	stem cells	bone marrow			pAMP10
L0534	Chromosome 7 Fetal Brain cDNA Library	brain	brain			pAMP10
L0535	NCI CGAP_Pr5	infiltrating ductal carcinoma	breast			pAMP10
L0539	Chromosome 7 Placental cDNA Library		placenta			pAMP10
L0540	NCI CGAP_Pr10	invasive prostate tumor	prostate			pAMP10
L0542	NCI CGAP_Pr11	normal prostatic epithelial cells	prostate			pAMP10
L0547	NCI CGAP_Pr16	tumor	prostate			pAMP10
L0549	NCI CGAP_HN10	carcinoma in situ from retromolar trigone				pAMP10
L0550	NCI CGAP_HN9	normal squamous epithelium from retromolar trigone				pAMP10
L0559	NCI CGAP_Ov39	papillary serous ovarian metastasis	ovary			pAMP10
L0561	NCI CGAP_HN11	normal squamous epithelium	tongue			pAMP10
L0562	Chromosome 7 HeLa cDNA Library			HeLa cell line; ATCC		pAMP10
L0563	Human Bone Marrow Stromal Fibroblast	bone marrow				pBluescript
L0564	Jia bone marrow stroma	bone marrow stroma				pBluescript
L0565	Normal Human Trabecular Bone Cells	Bone	Hip			pBluescript
L0581	Stratagene liver (#937224)		liver			pBluescript SK
L0587	Stratagene colon HT29 (#937221)					pBluescript SK-
L0588	Stratagene endothelial cell 937223					pBluescript SK-

L0589	Stratagene fetal retina 937202						pBluescript SK-
L0590	Stratagene fibroblast (#937212)						pBluescript SK-
L0591	Stratagene HeLa cell s3 937216						pBluescript SK-
L0592	Stratagene hNT neuron (#937233)						pBluescript SK-
L0593	Stratagene neuroepithelium (#937231)						pBluescript SK-
L0594	Stratagene neuroepithelium NT2RAMI 937234						pBluescript SK-
L0595	Stratagene NT2 neuronal precursor 937230	neuroepithelial cells	brain				pBluescript SK-
L0596	Stratagene colon (#937204)		colon				pBluescript SK-
L0597	Stratagene corneal stroma (#937222)		cornea				pBluescript SK-
L0598	Morton Fetal Cochlea	cochlea	ear				pBluescript SK-
L0599	Stratagene lung (#937210)		lung				pBluescript SK-
L0600	Weizmann Olfactory Epithelium	olfactory epithelium	nose				pBluescript SK-
L0601	Stratagene pancreas (#937208)		pancreas				pBluescript SK-
L0602	Pancreatic Islet	pancreatic islet	pancreas				pBluescript SK-
L0603	Stratagene placenta (#937225)		placenta				pBluescript SK-
L0604	Stratagene muscle 937209	muscle	skeletal muscle				pBluescript SK-
L0605	Stratagene fetal spleen (#937205)	fetal spleen	spleen				pBluescript SK-
L0606	NCI CGAP Lym5	follicular lymphoma	lymph node				pBluescript SK-
L0607	NCI CGAP Lym6	mantle cell lymphoma	lymph node				pBluescript SK-
L0608	Stratagene lung carcinoma 937218	lung carcinoma	lung			NCI-H69	pBluescript SK-

L0612	Schiller oligodendroglioma	oligodendroglioma	brain			pBluescript SK- (Stratagene)
L0615	22 week old human fetal liver cDNA library					pBluescriptII SK(-)
L0619	Chromosome 9 exon II					pBluescriptIIKS+
L0622	HM1					pcDNAII (Invitrogen)
L0623	HM3	pectoral muscle (after mastectomy)				pcDNAII (Invitrogen)
L0625	NCI CGAP AR1	bulk alveolar tumor				pCMV-SPORT2
L0626	NCI CGAP GC1	bulk germ cell seminoma				pCMV-SPORT2
L0627	NCI CGAP Co1	bulk tumor	colon			pCMV-SPORT2
L0628	NCI CGAP Ov1	ovary bulk tumor	ovary			pCMV-SPORT2
L0629	NCI CGAP_Mel3	metastatic melanoma to bowel	bowel (skin primary)			pCMV-SPORT4
L0630	NCI CGAP CNS1	substantia nigra	brain			pCMV-SPORT4
L0631	NCI CGAP Br7		breast			pCMV-SPORT4
L0635	NCI CGAP_PNS1	dorsal root ganglion	peripheral nervous system			pCMV-SPORT4
L0636	NCI CGAP_Pit1	four pooled pituitary adenomas	brain			pCMV-SPORT6
L0637	NCI CGAP_Bm53	three pooled meningiomas	brain			pCMV-SPORT6
L0638	NCI CGAP_Bm35	tumor, 5 pooled (see description)	brain			pCMV-SPORT6
L0639	NCI CGAP_Bm52	tumor, 5 pooled (see description)	brain			pCMV-SPORT6
L0640	NCI CGAP_Br18	four pooled high-grade tumors, including two primary	breast			pCMV-SPORT6
L0641	NCI CGAP_Co17	juvenile granulosa tumor	colon			pCMV-SPORT6
L0642	NCI CGAP_Co18	moderately differentiated adenocarcinoma	colon			pCMV-SPORT6
L0643	NCI CGAP_Co19	moderately differentiated adenocarcinoma	colon			pCMV-SPORT6
L0644	NCI CGAP_Co20	moderately differentiated adenocarcinoma	colon			pCMV-SPORT6

L0645	NCI_CGAP_Co21	moderately differentiated adenocarcinoma	colon			pCMV-SPORT6
L0646	NCI_CGAP_Co14	moderately-differentiated adenocarcinoma	colon			pCMV-SPORT6
L0647	NCI_CGAP_Sar4	five pooled sarcomas, including myxoid liposarcoma	connective tissue			pCMV-SPORT6
L0648	NCI_CGAP_Eso2	squamous cell carcinoma	esophagus			pCMV-SPORT6
L0649	NCI_CGAP_GUI	2 pooled high-grade transitional cell tumors	genitourinary tract			pCMV-SPORT6
L0650	NCI_CGAP_Kid13	2 pooled Wilms' tumors, one primary and one metast	kidney			pCMV-SPORT6
L0651	NCI_CGAP_Kid8	renal cell tumor	kidney			pCMV-SPORT6
L0652	NCI_CGAP_Lu27	four pooled poorly-differentiated adenocarcinomas	lung			pCMV-SPORT6
L0653	NCI_CGAP_Lu28	two pooled squamous cell carcinomas	lung			pCMV-SPORT6
L0654	NCI_CGAP_Lu31		lung, cell line			pCMV-SPORT6
L0655	NCI_CGAP_Lym12	lymphoma, follicular mixed small and large cell	lymph node			pCMV-SPORT6
L0656	NCI_CGAP_Ov38	normal epithelium	ovary			pCMV-SPORT6
L0657	NCI_CGAP_Ov23	tumor, 5 pooled (see description)	ovary			pCMV-SPORT6
L0658	NCI_CGAP_Ov35	tumor, 5 pooled (see description)	ovary			pCMV-SPORT6
L0659	NCI_CGAP_Pan1	adenocarcinoma	pancreas			pCMV-SPORT6
L0661	NCI_CGAP_Mel15	malignant melanoma, metastatic to lymph node	skin			pCMV-SPORT6
L0662	NCI_CGAP_Gas4	poorly differentiated adenocarcinoma with signet r	stomach			pCMV-SPORT6
L0663	NCI_CGAP_Ur2	moderately-differentiated endometrial adenocarcino	uterus			pCMV-SPORT6

L0664	NCI_CGAP_Ut3	poorly-differentiated endometrial adenocarcinoma,	uterus			pCMV-SPORT6
L0665	NCI_CGAP_Ut4	serous papillary carcinoma, high grade, 2 pooled t	uterus			pCMV-SPORT6
L0666	NCI_CGAP_Ut1	well-differentiated endometrial adenocarcinoma, 7	uterus			pCMV-SPORT6
L0667	NCI_CGAP_CML1	myeloid cells, 18 pooled CML cases, BCR/ABL rearra	whole blood			pCMV-SPORT6
L0684	Stanley Frontal SB pool 1	frontal lobe (see description)	brain			pCR2.1-TOPO (Invitrogen)
L0710	NIH_MGC_7	small cell carcinoma	lung	MGC3		pOTB7
L0717	Gessler Wilms tumor					pSPORT1
L0718	Testis 5					pSPORT1
L0731	Soares_pregnant_uterus_N bHPU		uterus			pT7T3-Pac
L0738	Human colorectal cancer					pT7T3D
L0740	Soares melanocyte 2NbHM	melanocyte				pT7T3D (Pharmacia) with a modified polylinker
L0741	Soares adult brain N2b4HB55Y		brain			pT7T3D (Pharmacia) with a modified polylinker
L0742	Soares adult brain N2b5HB55Y		brain			pT7T3D (Pharmacia) with a modified polylinker
L0743	Soares breast 2NbHBst		breast			pT7T3D (Pharmacia) with a modified polylinker
L0744	Soares breast 3NbHBst		breast			pT7T3D (Pharmacia) with a modified polylinker
L0745	Soares retina N2b4HR	retina	eye			pT7T3D (Pharmacia) with a modified polylinker
L0746	Soares retina N2b5HR	retina	eye			pT7T3D (Pharmacia) with a modified polylinker
L0747	Soares_fetal_heart_NbHH1 9W		heart			pT7T3D (Pharmacia) with a modified polylinker
L0748	Soares fetal liver spleen		Liver and			pT7T3D (Pharmacia) with a

	INFLS		Spleen		modified polylinker
L0749	Soares_fetal_liver_spleen_ INFLS S1		Liver and Spleen		pT7T3D (Pharmacia) with a modified polylinker
L0750	Soares_fetal_lung_NbHL1 9W		lung		pT7T3D (Pharmacia) with a modified polylinker
L0751	Soares ovary tumor NbHOT	ovarian tumor	ovary		pT7T3D (Pharmacia) with a modified polylinker
L0752	Soares_parathyroid_tumor NbHPA	parathyroid tumor	parathyroid gland		pT7T3D (Pharmacia) with a modified polylinker
L0753	Soares_pineal_gland_N3H PG		pineal gland		pT7T3D (Pharmacia) with a modified polylinker
L0754	Soares placenta Nb2HP		placenta		pT7T3D (Pharmacia) with a modified polylinker
L0755	Soares_placenta_8to9week s_2NbHP8to9W		placenta		pT7T3D (Pharmacia) with a modified polylinker
L0756	Soares_multiple_sclerosis_ 2NbHMSP	multiple sclerosis lesions			pT7T3D (Pharmacia) with a modified polylinker V TYPE
L0757	Soares_senescent_fibroblas ts_NbHSF	senescent fibroblast			pT7T3D (Pharmacia) with a modified polylinker V TYPE
L0758	Soares_testis_NHT				pT7T3D-Pac (Pharmacia) with a modified polylinker
L0759	Soares_total_fetus_Nb2HF 8 9w				pT7T3D-Pac (Pharmacia) with a modified polylinker
L0761	NCI_CGAP_CLL1	B-cell, chronic lymphocytic leukemia			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0762	NCI_CGAP_Br1.1	breast			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0763	NCI_CGAP_Br2	breast			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0764	NCI_CGAP_Co3	colon			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0765	NCI_CGAP_Co4	colon			pT7T3D-Pac (Pharmacia) with a modified polylinker

L0766	NCI_CGAP_GCB1	germinal center B cell				pT7T3D-Pac (Pharmacia) with a modified polylinker
L0767	NCI_CGAP_GC3	pooled germ cell tumors				pT7T3D-Pac (Pharmacia) with a modified polylinker
L0768	NCI_CGAP_GC4	pooled germ cell tumors				pT7T3D-Pac (Pharmacia) with a modified polylinker
L0769	NCI_CGAP_Bm25	anaplastic oligodendroglioma	brain			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0770	NCI_CGAP_Bm23	glioblastoma (pooled)	brain			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0771	NCI_CGAP_Co8	adenocarcinoma	colon			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0772	NCI_CGAP_Co10	colon tumor RER+	colon			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0773	NCI_CGAP_Co9	colon tumor RER+	colon			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0774	NCI_CGAP_Kid3		kidney			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0775	NCI_CGAP_Kid5	2 pooled tumors (clear cell type)	kidney			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0776	NCI_CGAP_Lu5	carcinoid	lung			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0777	Soares_NhHMPu_S1	Pooled human melanocyte, fetal heart, and pregnant	mixed (see below)			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0779	Soares_NFL_T_GBC_S1		pooled			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0780	Soares_NSF_F8_9W_OT_PA_P_S1		pooled			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0782	NCI_CGAP_Pr21	normal prostate	prostate			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0783	NCI_CGAP_Pr22	normal prostate	prostate			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0784	NCI_CGAP_Lei2	leiomyosarcoma	soft tissue			pT7T3D-Pac (Pharmacia) with a modified polylinker

L0785	Barstead spleen HPLRB2			spleen			modified polylinker
L0786	Soares_NbHFB			whole brain			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0787	NCI_CGAP_Sub1						pT7T3D-Pac (Pharmacia) with a modified polylinker
L0788	NCI_CGAP_Sub2						pT7T3D-Pac (Pharmacia) with a modified polylinker
L0789	NCI_CGAP_Sub3						pT7T3D-Pac (Pharmacia) with a modified polylinker
L0790	NCI_CGAP_Sub4						pT7T3D-Pac (Pharmacia) with a modified polylinker
L0791	NCI_CGAP_Sub5						pT7T3D-Pac (Pharmacia) with a modified polylinker
L0792	NCI_CGAP_Sub6						pT7T3D-Pac (Pharmacia) with a modified polylinker
L0793	NCI_CGAP_Sub7						pT7T3D-Pac (Pharmacia) with a modified polylinker
L0794	NCI_CGAP_GC6		pooled germ cell tumors				pT7T3D-Pac (Pharmacia) with a modified polylinker
L0796	NCI_CGAP_Brm50		medulloblastoma	brain			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0800	NCI_CGAP_Co16		colon tumor, RER+	colon			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0803	NCI_CGAP_Kid11			kidney			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0804	NCI_CGAP_Kid12		2 pooled tumors (clear cell type)	kidney			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0805	NCI_CGAP_Lu24		carcinoid	lung			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0806	NCI_CGAP_Lu19		squamous cell carcinoma, poorly differentiated (4	lung			pT7T3D-Pac (Pharmacia) with a modified polylinker

L0807	NCI_CGAP_Ov18	fibrotheoma	ovary			pT7T3D-Pac (Pharmacia) with a modified polylinker
L0809	NCI_CGAP_Pr28		prostate			pT7T3D-Pac (Pharmacia) with a modified polylinker
L1819	HT0268		head neck			puc18
L2251	Human fetal lung	Fetal lung				
L2257	NIH_MGC_65	adenocarcinoma	colon			pCMV-SPORT6
L2258	NIH_MGC_67	retinoblastoma	eye			pCMV-SPORT6
L2259	NIH_MGC_68	large cell carcinoma	lung			pCMV-SPORT6
L2260	NIH_MGC_69	large cell carcinoma, undifferentiated	lung			pCMV-SPORT6
L2261	NIH_MGC_70	epithelioid carcinoma	pancreas			pCMV-SPORT6
L2262	NIH_MGC_72	melanotic melanoma	skin			pCMV-SPORT6
L2263	NIH_MGC_66	adenocarcinoma	ovary			pCMV-SPORT6
L2264	NIH_MGC_71	leiomyosarcoma	uterus			pCMV-SPORT6
L2265	NIH_MGC_39	adenocarcinoma	pancreas			pOTB7
L2270	Lupski_dorsal_root_ganglion	dorsal root ganglia				pCMV-SPORT6 (Life Technologies)
L2289	BT0757		breast			puc18
L2336	CT0428		colon			puc18
L2368	UT0041		uterus tumor			puc18
L2380	NN0068		nervous normal			puc18
L2381	NN0070		nervous normal			puc18
L2412	NN0136		nervous normal			puc18
L2482	HT0497		head neck			puc18
L2486	HT0527		head neck			puc18
L2497	HT0618		head neck			puc18
L2504	HT0636		head neck			puc18
L2543	HT0734		head neck			puc18
L2551	HT0744		head neck			puc18
L2653	NIH_MGC_58	hypernephroma	kidney			pDNR-LIB (Clontech)
L2654	NIH_MGC_9	adenocarcinoma cell line	ovary			pOTB7

L2655	NIH_MGC_55	from acute myelogenous leukemia	bone marrow			pDNR-LIB (Clontech)
L2669	NT0022		nervous tumor			puc18
L2673	NT0028		nervous tumor			puc18
L2706	NT0102		nervous tumor			puc18
L2744	FT0004		prostate tumor			puc18
L2758	FT0027		prostate tumor			puc18
L2759	FT0028		prostate tumor			puc18
L2767	FT0044		prostate tumor			puc18
L2877	AN0027		amion normal			puc18
L2904	BN0042		breast normal			puc18
L2910	BN0070		breast normal			puc18
L2915	BN0098		breast normal			puc18
L2985	BN0257		breast normal			puc18
L2991	BN0264		breast normal			puc18
L3019	BN0303		breast normal			puc18
L3280	FN0106		prostate normal			puc18
L3357	TN0034		testis normal			puc18
L3372	TN0068		testis normal			puc18
L3387	GKB	hepatocellular carcinoma				pBluescript sk(-)
L3388	GKC	hepatocellular carcinoma				pBluescript sk(-)
L3391	NIH_MGC_53	carcinoma, cell line	bladder			pDNR-LIB (Clontech)
L3499	HT0617		head neck			puc18
L3503	HT0870		head neck			puc18
L3560	TN0023		testis normal			puc18
L3585	TN0119		testis normal			puc18
L3642	ADA	Adrenal gland				pBluescript sk(-)
L3643	ADB	Adrenal gland				pBluescript sk(-)
L3644	ADC	Adrenal gland				pBluescript sk(-)
L3645	Cu	adrenal cortico adenoma for Cushing's syndrome				pBluescript sk(-)
L3646	DCA					pTriplEx2

L3649	DCB						pTriplEx2
L3651	FHTA						pTriplEx2
L3653	HTB						pBluescript sk(-)
L3655	HTC						pBluescript sk(-)
L3658	cdA						pTriplEx2
L3659	CB						pBluescript
L3663	NIH_MGC_60				prostate		pDNR-LIB (Clontech)
L3811	NPC				pituitary		pBluescript sk(-)
L3812	NPB				pituitary		pBluescript sk(-)
L3815	MDS				Bone marrow		pTriplEx2
L3816	HEMBA1				whole embryo, mainly head		pME18SFL3
L3817	HEMBB1				whole embryo, mainly body		pME18SFL3
L3818	MAMMA1				mammary gland		pME18SFL3
L3824	NT2RM2					NT2	pME18SFL3
L3825	NT2RM4					NT2	pME18SFL3
L3826	NT2RP1					NT2	pUC19FL3
L3827	NT2RP2					NT2	pME18SFL3
L3828	NT2RP3					NT2	pME18SFL3
L3832	PLACE1				placenta		pME18SFL3
L3833	PLACE2				placenta		pME18SFL3
L3872	NCI_CGAP_Skn1				skin, normal, 4 pooled sa		pCMV-SPORT6
L3904	NCI_CGAP_Brn64				glioblastoma with EGFR amplification	brain	pCMV-SPORT6
L3905	NCI_CGAP_Brn67				anaplastic oligodendroglioma with 1p/19q loss	brain	pCMV-SPORT6
L4500	NCI_CGAP_HN16				moderate to poorly differentiated invasive carcino	mouth	pAMPI10
L4559	NCI_CGAP_Thy3				follicular carcinoma	thyroid	pCMV-SPORT6
L4560	NCI_CGAP_Ut7				tumor	uterus	pCMV-SPORT6
L4747	NCI_CGAP_Brn41				oligodendroglioma	brain	pT7T3D-Pac (Pharmacia) with a

L5565	NCI_CGAP_Brm66	glioblastoma with probably TP53 mutation and witho	brain			modified polylinker pCMV-SPORT6
L5566	NCI_CGAP_Brm70	anaplastic oligodendroglioma	brain			pCMV-SPORT6.ccdB
L5568	NCI_CGAP_HN21	nasopharyngeal carcinoma	head/neck			pAMPI1
L5569	NCI_CGAP_HN17	normal epithelium	nasopharynx			pAMPI10
L5574	NCI_CGAP_HN19	normal epithelium	nasopharynx			pAMPI10
L5575	NCI_CGAP_Brm65	glioblastoma without EGFR amplification	brain			pCMV-SPORT6
L5622	NCI_CGAP_Skn3		skin			pCMV-SPORT6
L5623	NCI_CGAP_Skn4	squamous cell carcinoma	skin			pCMV-SPORT6

Description of Table 5

Table 5 provides a key to the OMIM reference identification numbers disclosed in Table 1B.1, column 9. OMIM reference identification numbers (Column 1) were derived from Online Mendelian Inheritance in Man (Online Mendelian Inheritance in Man, OMIM. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine, (Bethesda, MD) 2000. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>. Column 2 provides diseases associated with the cytologic band disclosed in Table 1B.1, column 8, as determined using the Morbid Map database.

Table 5

OMIM Reference	Description
104311	Alzheimer disease-3
106180	Myocardial infarction, susceptibility to
107300	Antithrombin III deficiency
108725	Atherosclerosis, susceptibility to
109150	Machado-Joseph disease
112410	Hypertension with brachydactyly
114400	Lynch cancer family syndrome II
114835	Monocyte carboxyesterase deficiency
116806	Colorectal cancer
116860	Cavernous angiomatous malformations
118800	Choreoathetosis, familial paroxysmal
120700	C3 deficiency
120950	C8 deficiency, type I
120960	C8 deficiency, type II
123000	Cranio metaphyseal dysplasia
123620	Cataract, cerulean, type 2, 601547
123660	Cataract, Coppock-like
125660	Myopathy, desminopathic
125660	Cardiomyopathy
126451	Schizophrenia, susceptibility to
126650	Chloride diarrhea, congenital, Finnish type, 214700
126650	Colon cancer
129900	EEC syndrome-1
131210	Atherosclerosis, susceptibility to
132700	Cylindromatosis
133171	[Erythrocytosis, familial], 133100
133450	Neuroepithelioma
133450	Ewing sarcoma
135700	Fibrosis of extraocular muscles, congenital, 1
136132	[Fish-odor syndrome], 602079
138079	Hyperinsulinism, familial, 602485
138079	MODY, type 2, 125851
138130	Hyperinsulinism-hyperammonemia syndrome
138140	Glucose transport defect, blood-brain barrier

138300	Hemolytic anemia due to glutathione reductase deficiency
138700	[Apolipoprotein H deficiency]
139250	Isolated growth hormone deficiency, Illig type with absent GH and Kowarski type with bioinactive GH
143890	Hypercholesterolemia, familial
145001	Hyperparathyroidism-jaw tumor syndrome
147670	Rabson-Mendenhall syndrome
147670	Diabetes mellitus, insulin-resistant, with acanthosis nigricans
147670	Leprechaunism
150200	[Placental lactogen deficiency]
150250	Larsen syndrome, autosomal dominant
151440	Leukemia, T-cell acute lymphoblastoid
153880	Macular dystrophy, dominant cystoid
154275	Malignant hyperthermia susceptibility 2
154276	Malignant hyperthermia susceptibility 3
157640	PEO with mitochondrial DNA deletions, type 1
157900	Moebius syndrome
164500	Spinocerebellar ataxia-7
164953	Liposarcoma
165240	Pallister-Hall syndrome, 146510
165240	Postaxial polydactyly type A1, 174200
165240	Greig cephalopolysyndactyly syndrome, 175700
168468	Metaphyseal chondrodysplasia, Murk Jansen type, 156400
168470	Humoral hypercalcemia of malignancy
170500	Myotonia congenita, atypical acetazolamide-responsive
170500	Paramyotonia congenita, 168300
170500	Hyperkalemic periodic paralysis
172490	Phosphorylase kinase deficiency of liver and muscle, 261750
173360	Thrombophilia due to excessive plasminogen activator inhibitor
173360	Hemorrhagic diathesis due to PAI1 deficiency
173610	Platelet alpha/delta storage pool deficiency
174900	Polyposis, juvenile intestinal
176960	Pituitary tumor, invasive
178300	Ptosis, hereditary congenital, 1
180104	Retinitis pigmentosa-9
180105	Retinitis pigmentosa-10
180297	Anemia, hemolytic, Rh-null, suppressor type, 268150
181430	Scapuloperoneal syndrome, myopathic type
182280	Small-cell cancer of lung
182600	Spastic paraplegia-3A
187040	Leukemia-1, T-cell acute lymphoblastic
190900	Colorblindness, tritan
192340	Diabetes insipidus, neurohypophyseal, 125700
193500	Rhabdomyosarcoma, alveolar, 268220
193500	Waardenburg syndrome, type I
193500	Waardenburg syndrome, type III, 148820
193500	Craniofacial-deafness-hand syndrome, 122880
200990	Acrocallosal syndrome
201460	Acyl-CoA dehydrogenase, long chain, deficiency of
203300	Hermansky-Pudlak syndrome
203740	Alpha-ketoglutarate dehydrogenase deficiency
205100	Amyotrophic lateral sclerosis, juvenile

212138	Carnitine-acylcarnitine translocase deficiency
219800	Cystinosis, nephropathic
222800	Hemolytic anemia due to bisphosphoglycerate mutase deficiency
231670	Glutaricaciduria, type I
232300	Glycogen storage disease II
233700	Chronic granulomatous disease due to deficiency of NCF-1
234200	Neurodegeneration with brain iron accumulation
236730	Urofacial syndrome
237300	Carbamoylphosphate synthetase I deficiency
240400	Scurvy
245200	Krabbe disease
246900	Lipoamide dehydrogenase deficiency
248611	Maple syrup urine disease, type Ib
249000	Meckel syndrome
249270	Thiamine-responsive megaloblastic anemia
251000	Methylmalonicaciduria, mutase deficiency type
252900	Sanfilippo syndrome, type A
253250	Mulibrey nanism
257220	Niemann-Pick disease, type C
257220	Niemann-Pick disease, type D, 257250
261670	Myopathy due to phosphoglycerate mutase deficiency
262000	Bjornstad syndrome
263200	Polycystic kidney disease, autosomal recessive
277730	Wernicke-Korsakoff syndrome, susceptibility to
600079	Colon cancer
600101	Deafness, autosomal dominant 2
600163	Long QT syndrome-3
600185	Pancreatic cancer
600185	Breast cancer 2, early onset
600211	Cleidocranial dysplasia, 119600
600266	Resistance/susceptibility to TB, etc.
600276	Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy, 125310
600512	Epilepsy, partial
600650	Myopathy due to CPT II deficiency, 255110
600650	CPT deficiency, hepatic, type II, 600649
600698	Salivary adenoma
600698	Uterine leiomyoma
600698	Lipoma
600698	Lipomatosis, mutiple, 151900
600701	Lipoma
600722	Ceroid lipofuscinosis, neuronal, variant juvenile type, with granular osmiophilic deposits
600722	Ceroid lipofuscinosis, neuronal-1, infantile, 256730
600808	Enuresis, nocturnal, 2
600850	Schizophrenia disorder-4
600882	Charcot-Marie-Tooth neuropathy-2B
600957	Persistent Mullerian duct syndrome, type I, 261550
600968	Gitelman syndrome, 263800
600971	Deafness, autosomal recessive 6
601208	Insulin-dependent diabetes mellitus-11
601226	Progressive external ophthalmoplegia, type 2

601277	Ichthyosis, lamellar, type 2
601649	Blepharophimosis, epicanthus inversus, and ptosis, type 2
601652	Glaucoma 1A, primary open angle, juvenile-onset, 137750
601669	Hirschsprung disease, one form
601690	Platelet-activating factor acetylhydrolase deficiency
601728	Bannayan-Zonana syndrome, 153480
601728	Cowden disease, 158350
601728	Endometrial carcinoma
601728	Lhermitte-Duclos syndrome
601843	Hypothyroidism, congenital, 274400
602116	Glioma
602136	Refsum disease, infantile, 266510
602136	Zellweger syndrome-1, 214100
602136	Adrenoleukodystrophy, neonatal, 202370
602447	Coronary artery disease, susceptibility to
602568	Homocystinuria-megaloblastic anemia, cbl E type, 236270
602629	Dystonia-6, torsion

Mature Polypeptides

The present invention also encompasses mature forms of a polypeptide having the amino acid sequence of SEQ ID NO:Y and/or the amino acid sequence encoded by the cDNA in a deposited clone. Polynucleotides encoding the mature forms (such as, for example, the polynucleotide sequence in SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone) are also encompassed by the invention. Moreover, fragments or variants of these polypeptides (such as, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to these polypeptides, or polypeptides encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of the polynucleotide encoding these polypeptides) are also encompassed by the invention. In preferred embodiments, these fragments or variants retain one or more functional activities of the full-length or mature form of the polypeptide (e.g., biological activity (such as, for example, activity useful in detecting, preventing, diagnosing, prognosticating, treating, and/or ameliorating allergic and/or asthmatic disorders), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an anti-polypeptide of the invention antibody), immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention). Antibodies that bind the polypeptides of the invention, and polynucleotides encoding these polypeptides are also encompassed by the invention.

According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence that is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases,

cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

5 Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, *Virus Res.* 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, *Nucleic Acids Res.* 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site,
10 typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, *supra*.) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

 In the present case, the deduced amino acid sequence of the secreted polypeptide was
15 analyzed by a computer program called SignalP (Henrik Nielsen et al., *Protein Engineering* 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis of the amino acid sequences of the secreted proteins described herein by this program provided the results shown in Table 1A.

20 In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the predicted mature form of the polypeptide as delineated in columns 14 and 15 of Table 1A. Moreover, fragments or variants of these polypeptides (such as, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to these polypeptides, or polypeptides encoded by a polynucleotide that hybridizes under stringent
25 conditions to the complementary strand of the polynucleotide encoding these polypeptides) are also encompassed by the invention. In preferred embodiments, these fragments or variants retain one or more functional activities of the full-length or mature form of the polypeptide (e.g., biological activity (such as, for example, activity useful in detecting, preventing, diagnosing, prognosticating, treating, and/or ameliorating allergic and/or asthmatic disorders), antigenicity
30 (ability to bind, or compete with a polypeptide of the invention for binding, to an anti-polypeptide of the invention antibody), immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention). Antibodies that bind the polypeptides of the invention, and polynucleotides encoding these polypeptides are also
35 encompassed by the invention.

 Polynucleotides encoding proteins comprising, or consisting of, the predicted mature form of polypeptides of the invention (e.g., polynucleotides having the sequence of SEQ ID NO: X

(Table 1A, column 4), the sequence delineated in columns 7 and 8 of Table 1A, and a sequence encoding the mature polypeptide delineated in columns 14 and 15 of Table 1A (e.g., the sequence of SEQ ID NO:X encoding the mature polypeptide delineated in columns 14 and 15 of Table 1)) are also encompassed by the invention, as are fragments or variants of these polynucleotides (such as, fragments as described herein, polynucleotides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to these polynucleotides, and nucleic acids which hybridizes under stringent conditions to the complementary strand of the polynucleotide).

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence shown in SEQ ID NO:Y which have an N-terminus beginning within 15 residues of the predicted cleavage point (i.e., having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 more or less contiguous residues of SEQ ID NO:Y at the N-terminus when compared to the predicted mature form of the polypeptide (e.g., the mature polypeptide delineated in columns 14 and 15 of Table 1). Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as described below). These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Polynucleotide and Polypeptide Variants

The present invention is also directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X or the complementary strand thereto, nucleotide sequences encoding the polypeptide of SEQ ID NO:Y, the nucleotide sequence of SEQ ID NO:X that encodes the polypeptide sequence as defined in columns 13 and 14 of Table 1A, nucleotide sequences encoding the polypeptide sequence as defined in columns 13 and 14 of Table 1A, the nucleotide sequence of SEQ ID NO:X encoding the polypeptide sequence as defined in Table 1B, nucleotide sequences encoding the polypeptide as defined in Table 1B, the nucleotide sequence as defined in columns 8 and 9 of Table 2, nucleotide sequences encoding the polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2, the nucleotide sequence as defined in column 6 of Table 1C, nucleotide sequences encoding the polypeptide encoded by the

nucleotide sequence as defined in column 6 of Table 1C, the cDNA sequence contained in ATCC Deposit No:Z, nucleotide sequences encoding the polypeptide encoded by the cDNA sequence contained in ATCC Deposit No:Z, and/or nucleotide sequences encoding a mature (secreted) polypeptide encoded by the cDNA sequence contained in ATCC Deposit No:Z.

5 The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y, the polypeptide as defined in columns 13 and 14 of Table 1A, the polypeptide sequence as defined in Table 1B, a polypeptide sequence encoded by the polynucleotide sequence in SEQ ID NO:X, a polypeptide sequence encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2, a polypeptide sequence encoded by the nucleotide sequence as
10 defined in column 6 of Table 1C, a polypeptide sequence encoded by the complement of the polynucleotide sequence in SEQ ID NO:X, the polypeptide sequence encoded by the cDNA sequence contained in ATCC Deposit No:Z and/or a mature (secreted) polypeptide encoded by the cDNA sequence contained in ATCC Deposit No:Z.

 "Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or
15 polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

 Thus, one aspect of the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide having a nucleotide sequence selected from the
20 group consisting of: (a) a nucleotide sequence described in SEQ ID NO:X or contained in the cDNA sequence of ATCC Deposit No:Z; (b) a nucleotide sequence in SEQ ID NO:X or the cDNA in ATCC Deposit No:Z which encodes the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in ATCC Deposit No:Z; (c) a nucleotide sequence in SEQ ID NO:X or the cDNA in ATCC Deposit No:Z which encodes a mature
25 polypeptide (i.e., a secreted polypeptide (e.g., as delineated in columns 14 and 15 of Table 1A)); (d) a nucleotide sequence in SEQ ID NO:X or the cDNA sequence of ATCC Deposit No:Z, which encodes a biologically active fragment of a polypeptide; (e) a nucleotide sequence in SEQ ID NO:X or the cDNA sequence of ATCC Deposit No:Z, which encodes an antigenic fragment of a polypeptide; (f) a nucleotide sequence encoding a polypeptide comprising the complete amino
30 acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in ATCC Deposit No:Z; (g) a nucleotide sequence encoding a mature polypeptide of the amino acid sequence of SEQ ID NO:Y (i.e., a secreted polypeptide (e.g., as delineated in columns 14 and 15 of Table 1A)) or a mature polypeptide of the amino acid sequence encoded by the cDNA in ATCC Deposit No:Z; (h) a nucleotide sequence encoding a biologically active fragment of a polypeptide
35 having the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in ATCC Deposit No:Z; (i) a nucleotide sequence encoding an antigenic fragment of a polypeptide having the complete amino acid sequence of SEQ ID NO:Y or the

complete amino acid sequence encoded by the cDNA in ATCC Deposit No:Z; and (j) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), or (i) above.

5 The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, identical to, for example, any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i), or (j) above, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the nucleotide coding sequence of the cDNA contained in ATCC Deposit No:Z or the complementary strand thereto, a nucleotide sequence encoding the
10 polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding a polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X, a polypeptide sequence encoded by the complement of the polynucleotide sequence in SEQ ID NO:X, a nucleotide sequence encoding the polypeptide encoded by the cDNA contained in ATCC Deposit No:Z, the nucleotide coding sequence in SEQ ID NO:X as defined in columns 8 and 9 of Table 2 or the complementary strand thereto, a
15 nucleotide sequence encoding the polypeptide encoded by the nucleotide sequence in SEQ ID NO:X as defined in columns 8 and 9 of Table 2 or the complementary strand thereto, the nucleotide coding sequence in SEQ ID NO:B as defined in column 6 of Table 1C or the complementary strand thereto, a nucleotide sequence encoding the polypeptide encoded by the nucleotide sequence in SEQ ID NO:B as defined in column 6 of Table 1C or the complementary
20 strand thereto, the nucleotide sequence in SEQ ID NO:X encoding the polypeptide sequence as defined in Table 1B or the complementary strand thereto, nucleotide sequences encoding the polypeptide as defined in Table 1B or the complementary strand thereto, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polynucleotides that hybridize to the complement of these nucleic acid molecules under stringent
25 hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides and nucleic acids.

In a preferred embodiment, the invention encompasses nucleic acid molecules which comprise, or alternatively, consist of a polynucleotide which hybridizes under stringent hybridization conditions, or alternatively, under lower stringency conditions, to a polynucleotide in
30 (a), (b), (c), (d), (e), (f), (g), (h), or (i), above, as are polypeptides encoded by these polynucleotides. In another preferred embodiment, polynucleotides which hybridize to the complement of these nucleic acid molecules under stringent hybridization conditions, or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

35 In another embodiment, the invention provides a purified protein comprising, or alternatively consisting of, a polypeptide having an amino acid sequence selected from the group consisting of: (a) the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid

sequence encoded by the cDNA in ATCC Deposit No:Z; (b) the amino acid sequence of a mature (secreted) form of a polypeptide having the amino acid sequence of SEQ ID NO:Y (e.g., as delineated in columns 14 and 15 of Table 1A) or a mature form of the amino acid sequence encoded by the cDNA in ATCC Deposit No:Z mature; (c) the amino acid sequence of a biologically active fragment of a polypeptide having the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in ATCC Deposit No:Z; and (d) the amino acid sequence of an antigenic fragment of a polypeptide having the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in ATCC Deposit No:Z.

The present invention is also directed to proteins which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, identical to, for example, any of the amino acid sequences in (a), (b), (c), or (d), above, the amino acid sequence shown in SEQ ID NO:Y, the amino acid sequence encoded by the cDNA contained in ATCC Deposit No:Z, the amino acid sequence of the polypeptide encoded by the nucleotide sequence in SEQ ID NO:X as defined in columns 8 and 9 of Table 2, the amino acid sequence of the polypeptide encoded by the nucleotide sequence in SEQ ID NO:B as defined in column 6 of Table 1C, the amino acid sequence as defined in Table 1B, an amino acid sequence encoded by the nucleotide sequence in SEQ ID NO:X, and an amino acid sequence encoded by the complement of the polynucleotide sequence in SEQ ID NO:X. Fragments of these polypeptides are also provided (e.g., those fragments described herein). Further proteins encoded by polynucleotides that hybridize to the complement of the nucleic acid molecules encoding these amino acid sequences under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are the polynucleotides encoding these proteins.

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence referred to in Table 1B or 2 as the ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred

method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is expressed as percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence that are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to

a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence of a polypeptide referred to in Table 1A (e.g., the amino acid sequence delineated in columns 14 and 15) or a fragment thereof, Table 1B.1 (e.g., the amino acid sequence identified in column 6) or a fragment thereof, Table 2 (e.g., the amino acid sequence of the polypeptide encoded by the polynucleotide sequence defined in columns 8 and 9 of Table 2) or a fragment thereof, the amino acid sequence of the polypeptide encoded by the polynucleotide sequence in SEQ ID NO:B as defined in column 6 of Table 1C or a fragment thereof, the amino acid sequence of the polypeptide encoded by the nucleotide sequence in SEQ ID NO:X or a fragment thereof, or the amino acid sequence of the polypeptide encoded by cDNA contained in ATCC Deposit No:Z, or a fragment thereof, the amino acid sequence of a mature (secreted) polypeptide encoded by cDNA contained in ATCC Deposit No:Z, or a fragment thereof, can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci.6:237-245 (1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is expressed as percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the

number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence that are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The polynucleotide variants of the invention may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations that produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, polypeptide variants in which less than 50, less than 40, less than 30, less than 20, less than 10, or 5-50, 5-25, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985)). These allelic variants can vary at either

the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants
5 may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the polypeptide of the present invention without substantial loss of biological function. As an example, Ron et al. (J. Biol. Chem. 268: 2984-2988 (1993)) reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues.
10 Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol.
15 Chem. 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." In fact, only
20 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce
25 and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants that show a biological or functional activity of the polypeptides of the invention (such as, for example, activity useful in detecting, preventing, diagnosing, prognosticating, treating, and/or ameliorating allergic and/or
asthmatic disorders). Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity.

35 The present application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein, (e.g., encoding a polypeptide having the amino acid sequence of an N and/or C terminal deletion),

irrespective of whether they encode a polypeptide having functional activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having functional activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having functional activity include, inter alia, (1) isolating a gene or allelic or splice variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the gene, as described in Verma et al., Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York (1988); (3) Northern Blot analysis for detecting mRNA expression in specific tissues (e.g., normal or diseased tissues); and (4) *in situ* hybridization (e.g., histochemistry) for detecting mRNA expression in specific tissues (e.g., normal or diseased tissues).

Preferred, however, are nucleic acid molecules having sequences at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein, which do, in fact, encode a polypeptide having functional activity. By a polypeptide having "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) protein and/or a mature (secreted) protein of the invention. Such functional activities include, but are not limited to, biological activity (such as, for example, activity useful in detecting, preventing, diagnosing, prognosticating, treating, and/or ameliorating allergic and/or asthmatic diseases and disorders), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an anti-polypeptide of the invention antibody), immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention.

The functional activity of the polypeptides, and fragments, variants and derivatives of the invention, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with a full-length polypeptide of the present invention for binding to an anti-polypeptide antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary

antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

5 In another embodiment, where a ligand is identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky et al., Microbiol. Rev. 59:94-123 (1995). In another embodiment, the ability of physiological correlates of a polypeptide of the present invention to bind to a substrate(s) of the polypeptide of
10 the invention can be routinely assayed using techniques known in the art.

In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the present invention and fragments, variants and derivatives thereof to elicit polypeptide related biological activity (either *in vitro* or *in vivo*). Other methods will be known to the skilled artisan and are within the scope of the
15 invention.

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to, for example, the nucleic acid sequence of the cDNA contained in ATCC Deposit No:Z, the nucleic acid sequence referred
20 to in Table 1B (SEQ ID NO:X), the nucleic acid sequence disclosed in Table 1A (e.g., the nucleic acid sequence delineated in columns 7 and 8), the nucleic acid sequence disclosed in Table 2 (e.g., the nucleic acid sequence delineated in columns 8 and 9) or fragments thereof, will encode polypeptides "having functional activity." In fact, since degenerate variants of any of these nucleotide sequences all encode the same polypeptide, in many instances, this will be clear to the
25 skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having functional activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second
30 aliphatic amino acid), as further described below.

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to
35 change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species,

conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. See Cunningham and Wells, *Science* 244:1081-1085 (1989). The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitutions with one or more of the amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, serum albumin (preferably human serum albumin) or a fragment thereof, or leader or secretory sequence, or a sequence facilitating purification, or (v) fusion of the polypeptide with another compound, such as albumin (including but not limited to recombinant albumin (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. See

Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).

A further embodiment of the invention relates to polypeptides which comprise the amino acid sequence of a polypeptide having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions from a polypeptide sequence disclosed herein. Of course it is highly preferable for a polypeptide to have an amino acid sequence which, for example, comprises the amino acid sequence of a polypeptide of SEQ ID NO:Y, the amino acid sequence of the mature (e.g., secreted) polypeptide of SEQ ID NO:Y, an amino acid sequence encoded by SEQ ID NO:X, an amino acid sequence encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, an amino acid sequence encoded by the complement of SEQ ID NO:X, an amino acid sequence encoded by cDNA contained in ATCC Deposit No:Z, and/or the amino acid sequence of a mature (secreted) polypeptide encoded by cDNA contained in ATCC Deposit No:Z, or a fragment thereof, which contains, in order of ever-increasing preference, at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions.

In specific embodiments, the polypeptides of the invention comprise, or alternatively, consist of, fragments or variants of a reference amino acid sequence selected from: (a) the amino acid sequence of SEQ ID NO:Y or fragments thereof (e.g., the mature form and/or other fragments described herein); (b) the amino acid sequence encoded by SEQ ID NO:X or fragments thereof; (c) the amino acid sequence encoded by the complement of SEQ ID NO:X or fragments thereof; (d) the amino acid sequence encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2 or fragments thereof; and (e) the amino acid sequence encoded by cDNA contained in ATCC Deposit No:Z or fragments thereof; wherein the fragments or variants have 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, amino acid residue additions, substitutions, and/or deletions when compared to the reference amino acid sequence. In preferred embodiments, the amino acid substitutions are conservative. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Polynucleotide and Polypeptide Fragments

The present invention is also directed to polynucleotide fragments of the polynucleotides (nucleic acids) of the invention. In the present invention, a "polynucleotide fragment" refers to a polynucleotide having a nucleic acid sequence which, for example: is a portion of the cDNA contained in ATCC Deposit No:Z or the complementary strand thereto; is a portion of the polynucleotide sequence encoding the polypeptide encoded by the cDNA contained in ATCC Deposit No:Z or the complementary strand thereto; is a portion of the polynucleotide sequence

encoding the mature (secreted) polypeptide encoded by the cDNA contained in ATCC Deposit No:Z or the complementary strand thereto; is a portion of a polynucleotide sequence encoding the mature amino acid sequence as defined in columns 14 and 15 of Table 1A or the complementary strand thereto; is a portion of a polynucleotide sequence encoding the amino acid sequence encoded by the region of SEQ ID NO:X as defined in columns 8 and 9 of Table 2 or the complementary strand thereto; is a portion of the polynucleotide sequence of SEQ ID NO:X as defined in columns 8 and 9 of Table 2 or the complementary strand thereto; is a portion of the polynucleotide sequence in SEQ ID NO:X or the complementary strand thereto; is a polynucleotide sequence encoding a portion of the polypeptide of SEQ ID NO:Y; is a polynucleotide sequence encoding a portion of a polypeptide encoded by SEQ ID NO:X; is a polynucleotide sequence encoding a portion of a polypeptide encoded by the complement of the polynucleotide sequence in SEQ ID NO:X; is a portion of a polynucleotide sequence encoding the amino acid sequence encoded by the region of SEQ ID NO:B as defined in column 6 of Table 1C or the complementary strand thereto; or is a portion of the polynucleotide sequence of SEQ ID NO:B as defined in column 6 of Table 1C or the complementary strand thereto.

The polynucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in ATCC Deposit No:Z, or the nucleotide sequence shown in SEQ ID NO:X or the complementary strand thereto. In this context "about" includes the particularly recited value or a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., at least 160, 170, 180, 190, 200, 250, 500, 600, 1000, or 2000 nucleotides in length) are also encompassed by the invention.

Moreover, representative examples of polynucleotide fragments of the invention comprise, or alternatively consist of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 601-650, 651-700, 701-750, 751-800, 801-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050, 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550, 3551-3600, 3601-3650, 3651-3700, 3701-3750, 3751-3800, 3801-3850, 3851-3900, 3901-

3950, 3951-4000, 4001-4050, 4051-4100, 4101-4150, 4151-4200, 4201-4250, 4251-4300, 4301-4350, 4351-4400, 4401-4450, 4451-4500, 4501-4550, 4551-4600, 4601-4650, 4651-4700, 4701-4750, 4751-4800, 4801-4850, 4851-4900, 4901-4950, 4951-5000, 5001-5050, 5051-5100, 5101-5150, 5151-5200, 5201-5250, 5251-5300, 5301-5350, 5351-5400, 5401-5450, 5451-5500, 5501-5550, 5551-5600, 5601-5650, 5651-5700, 5701-5750, 5751-5800, 5801-5850, 5851-5900, 5901-5950, 5951-6000, 6001-6050, 6051-6100, 6101-6150, 6151-6200, 6201-6250, 6251-6300, 6301-6350, 6351-6400, 6401-6450, 6451-6500, 6501-6550, 6551-6600, 6601-6650, 6651-6700, 6701-6750, 6751-6800, 6801-6850, 6851-6900, 6901-6950, 6951-7000, 7001-7050, 7051-7100, 7101-7150, 7151-7200, 7201-7250, 7251-7300 or 7301 to the end of SEQ ID NO:X, or the complementary strand thereto. In this context “about” includes the particularly recited range or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide that has a functional activity (e.g., biological activity; such as, for example, activity useful in detecting, preventing, diagnosing, prognosticating, treating, and/or ameliorating allergic and/or asthmatic diseases and disorders). More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to one or more of these polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

Further representative examples of polynucleotide fragments of the invention comprise, or alternatively consist of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 601-650, 651-700, 701-750, 751-800, 801-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050, 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550, 3551-3600, 3601-3650, 3651-3700, 3701-3750, 3751-3800, 3801-3850, 3851-3900, 3901-3950, 3951-4000, 4001-4050, 4051-4100, 4101-4150, 4151-4200, 4201-4250, 4251-4300, 4301-4350, 4351-4400, 4401-4450, 4451-4500, 4501-4550, 4551-4600, 4601-4650, 4651-4700, 4701-4750, 4751-4800, 4801-4850, 4851-4900, 4901-4950, 4951-5000, 5001-5050, 5051-5100, 5101-5150, 5151-5200, 5201-5250, 5251-5300, 5301-5350, 5351-5400, 5401-5450, 5451-5500, 5501-5550, 5551-5600, 5601-5650, 5651-5700, 5701-5750, 5751-5800, 5801-5850, 5851-5900, 5901-5950, 5951-6000, 6001-6050, 6051-6100, 6101-6150, 6151-6200, 6201-6250, 6251-6300, 6301-6350, 6351-6400, 6401-6450, 6451-6500, 6501-6550, 6551-6600, 6601-6650, 6651-6700, 6701-6750, 6751-6800, 6801-6850, 6851-6900, 6901-6950, 6951-7000, 7001-7050, 7051-7100, 7101-

7150, 7151-7200, 7201-7250, 7251-7300 or 7301 to the end of the cDNA sequence contained in ATCC Deposit No:Z, or the complementary strand thereto. In this context "about" includes the particularly recited range or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide that has a functional activity (e.g., biological activity). More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to one or more of these polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

Moreover, representative examples of polynucleotide fragments of the invention comprise, or alternatively consist of, a nucleic acid sequence comprising one, two, three, four, five, six, seven, eight, nine, ten, or more of the above described polynucleotide fragments of the invention in combination with a polynucleotide sequence delineated in Table 1C column 6. Additional, representative examples of polynucleotide fragments of the invention comprise, or alternatively consist of, a nucleic acid sequence comprising one, two, three, four, five, six, seven, eight, nine, ten, or more of the above described polynucleotide fragments of the invention in combination with a polynucleotide sequence that is the complementary strand of a sequence delineated in column 6 of Table 1C. In further embodiments, the above-described polynucleotide fragments of the invention comprise, or alternatively consist of, sequences delineated in Table 1C, column 6, and have a nucleic acid sequence which is different from that of the BAC fragment having the sequence disclosed in SEQ ID NO:B (see Table 1C, column 5). In additional embodiments, the above-described polynucleotide fragments of the invention comprise, or alternatively consist of, sequences delineated in Table 1C, column 6, and have a nucleic acid sequence which is different from that published for the BAC clone identified as BAC ID NO:A (see Table 1C, column 4). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated Table 1C, column 6, and have a nucleic acid sequence which is different from that contained in the BAC clone identified as BAC ID NO:A (see Table 1C, column 4). Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides and polypeptides are also encompassed by the invention.

In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more fragments of the sequences delineated in column 6 of Table 1C, and the polynucleotide sequence of SEQ ID NO:X (e.g., as defined in Table 1C, column 2) or fragments or variants thereof. Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention.

In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more fragments of the sequences delineated in column 6 of Table 1C which correspond to the same ATCC Deposit No:Z (see Table 1C, column 1), and the polynucleotide sequence of SEQ ID NO:X (e.g., as defined in Table 1A, 1B, or 1C) or fragments or variants thereof. Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention.

In further specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more fragments of the sequences delineated in the same row of column 6 of Table 1C, and the polynucleotide sequence of SEQ ID NO:X (e.g., as defined in Table 1A, 1B, or 1C) or fragments or variants thereof. Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention.

In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1C and the 5' 10 polynucleotides of the sequence of SEQ ID NO:X are directly contiguous. Nucleic acids that hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1C and the 5' 10 polynucleotides of a fragment or variant of the sequence of SEQ ID NO:X (e.g., as described herein) are directly contiguous. Nucleic acids which hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In further specific embodiments, polynucleotides of the invention comprise, or alternatively consist of a polynucleotide sequence in which the 3' 10 polynucleotides of a fragment or variant of the sequence of SEQ ID NO:X and the 5' 10 polynucleotides of the sequence of one

of the sequences delineated in column 6 of Table 1C are directly contiguous. Nucleic acids that hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In specific embodiments, polynucleotides of the invention comprise, or alternatively consist of a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1C and the 5' 10 polynucleotides of another sequence in column 6 are directly contiguous. In preferred embodiments, the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1C is directly contiguous with the 5' 10 polynucleotides of the next sequential exon delineated in Table 1C, column 6. Nucleic acids that hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of the amino acid sequence contained in SEQ ID NO:Y, is a portion of the mature form of SEQ ID NO:Y as defined in columns 14 and 15 of Table 1A, a portion of an amino acid sequence encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, is a portion of an amino acid sequence encoded by the polynucleotide sequence of SEQ ID NO:X, is a portion of an amino acid sequence encoded by the complement of the polynucleotide sequence in SEQ ID NO:X, is a portion of the amino acid sequence of a mature (secreted) polypeptide encoded by the cDNA contained in ATCC Deposit No:Z, and/or is a portion of an amino acid sequence encoded by the cDNA contained in ATCC Deposit No:Z. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 101-120, 121-140, 141-160, 161-180, 181-200, 201-220, 221-240, 241-260, 261-280, 281-300, 301-320, 321-340, 341-360, 361-380, 381-400, 401-420, 421-440, 441-460, 461-480, 481-500, 501-520, 521-540, 541-560, 561-580, 581-600, 601-620, 621-640, 641-660, 661-680, 681-700, 701-720, 721-740, 741-760,

761-780, 781-800, 801-820, 821-840, 841-860, 861-880, 881-900, 901-920, 921-940, 941-960, 961-980, 981-1000, 1001-1020, 1021-1040, 1041-1060, 1061-1080, 1081-1100, 1101-1120, 1121-1140, 1141-1160, 1161-1180, 1181-1200, 1201-1220, 1221-1240, 1241-1260, 1261-1280, 1281-1300, 1301-1320, 1321-1340, 1341-1360, 1361-1380, 1381-1400, 1401-1420, 1421-1440, or 1441 to the end of the coding region of cDNA and SEQ ID NO: Y. In a preferred embodiment, polypeptide fragments of the invention include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 101-120, 121-140, 141-160, 161-180, 181-200, 201-220, 221-240, 241-260, 261-280, 281-300, 301-320, 321-340, 341-360, 361-380, 381-400, 401-420, 421-440, 441-460, 461-480, 481-500, 501-520, 521-540, 541-560, 561-580, 581-600, 601-620, 621-640, 641-660, 661-680, 681-700, 701-720, 721-740, 741-760, 761-780, 781-800, 801-820, 821-840, 841-860, 861-880, 881-900, 901-920, 921-940, 941-960, 961-980, 981-1000, 1001-1020, 1021-1040, 1041-1060, 1061-1080, 1081-1100, 1101-1120, 1121-1140, 1141-1160, 1161-1180, 1181-1200, 1201-1220, 1221-1240, 1241-1260, 1261-1280, 1281-1300, 1301-1320, 1321-1340, 1341-1360, 1361-1380, 1381-1400, 1401-1420, 1421-1440, or 1441 to the end of the coding region of SEQ ID NO:Y. Moreover, polypeptide fragments of the invention may be at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, or ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities; such as, for example, activity useful in detecting, preventing, diagnosing, prognosticating, treating, and/or ameliorating allergic and/or asthmatic diseases and disorders; ability to multimerize; ability to bind a ligand; antigenic ability useful for production of polypeptide specific antibodies) may still be retained. For example, the ability of shortened muteins to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both.

For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

The present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide as defined in columns 14 and 15 of Table 1A, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X or the complement thereof, a polypeptide encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, a polypeptide encoded by the portion of SEQ ID NO:B as defined in column 6 of Table 1C, a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z, and/or a mature polypeptide encoded by the cDNA contained in ATCC Deposit No:Z). In particular, N-terminal deletions may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y, the mature (secreted) portion of SEQ ID NO:Y as defined in columns 14 and 15 of Table 1A, or the polypeptide encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2), and m is defined as any integer ranging from 2 to q-6. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, the mature (secreted) portion of SEQ ID NO:Y as defined in columns 14 and 15 of Table 1A, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X, a polypeptide encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, a polypeptide encoded by the portion of SEQ ID NO:B as defined in column 6 of Table 1C, a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z, and/or a mature polypeptide encoded by the cDNA contained in ATCC Deposit No:Z). In particular, C-terminal deletions may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of amino acid residue in a polypeptide of the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In addition, any of the N- or C-terminal deletions described above can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of a polypeptide encoded by SEQ ID NO:X (e.g., including, but not limited to, the preferred polypeptide disclosed as SEQ ID NO:Y, the mature (secreted) portion of SEQ ID NO:Y as defined in columns 14 and 15 of Table 1A, and the polypeptide encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2),

the cDNA contained in ATCC Deposit No:Z, and/or the complement thereof, where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities (e.g., biological activities such as, for example, activity useful in detecting, preventing, diagnosing, prognosticating, treating, and/or ameliorating allergic and/or asthmatic diseases and disorders; ability to multimerize; ability to bind a ligand; antigenic ability useful for production of polypeptide specific antibodies) may still be retained. For example the ability of the shortened mutein to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

The present application is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set forth herein. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Any polypeptide sequence encoded by, for example, the polynucleotide sequences set forth as SEQ ID NO:X or the complement thereof, (presented, for example, in Tables 1A and 2), the cDNA contained in ATCC Deposit No:Z, or the polynucleotide sequence as defined in column 6 of Table 1C, may be analyzed to determine certain preferred regions of the polypeptide. For example, the amino acid sequence of a polypeptide encoded by a polynucleotide sequence of SEQ ID NO:X (e.g., the polypeptide of SEQ ID NO:Y and the polypeptide encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2) or the cDNA contained in ATCC Deposit No:Z may be analyzed using the default parameters of the DNASTAR computer algorithm (DNASTAR, Inc., 1228 S. Park St., Madison, WI 53715 USA; <http://www.dnastar.com/>).

Polypeptide regions that may be routinely obtained using the DNASTAR computer algorithm include, but are not limited to, Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman alpha-regions, beta-regions, and turn-regions; Kyte-Doolittle hydrophilic regions and hydrophobic regions; Eisenberg alpha- and

beta-amphipathic regions; Karplus-Schulz flexible regions; Emini surface-forming regions; and Jameson-Wolf regions of high antigenic index. Among highly preferred polynucleotides of the invention in this regard are those that encode polypeptides comprising regions that combine several structural features, such as several (e.g., 1, 2, 3 or 4) of the features set out above.

5 Additionally, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Emini surface-forming regions, and Jameson-Wolf regions of high antigenic index (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) can routinely be used to determine polypeptide regions that exhibit a high degree of potential for antigenicity. Regions of
10 high antigenicity are determined from data by DNASTAR analysis by choosing values that represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

 Preferred polypeptide fragments of the invention are fragments comprising, or
15 alternatively, consisting of, an amino acid sequence that displays a functional activity (e.g. biological activity such as, for example, activity useful in detecting, preventing, diagnosing, prognosticating, treating, and/or ameliorating allergic and/or asthmatic diseases and disorders; ability to multimerize; ability to bind a ligand; antigenic ability useful for production of polypeptide specific antibodies) of the polypeptide sequence of which the amino acid sequence is a
20 fragment. By a polypeptide displaying a "functional activity" is meant a polypeptide capable of one or more known functional activities associated with a full-length protein, such as, for example, biological activity, antigenicity, immunogenicity, and/or multimerization, as described herein.

 Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of
25 the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

 In preferred embodiments, polypeptides of the invention comprise, or alternatively consist of, one, two, three, four, five or more of the antigenic fragments of the polypeptide of SEQ ID NO:Y, or portions thereof. Polynucleotides encoding these polypeptides are also encompassed by
30 the invention.

Epitopes and Antibodies

 The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of: the polypeptide sequence shown in SEQ ID NO:Y; a polypeptide sequence
35 encoded by SEQ ID NO:X or the complementary strand thereto; the polypeptide sequence encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2; the polypeptide sequence encoded by the portion of SEQ ID NO:B as defined in column 6 of Table 1C

or the complement thereto; the polypeptide sequence encoded by the cDNA contained in ATCC Deposit No:Z; or the polypeptide sequence encoded by a polynucleotide that hybridizes to the sequence of SEQ ID NO:X, the complement of the sequence of SEQ ID NO:X, the complement of a portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, or the cDNA sequence contained in ATCC Deposit No:Z under stringent hybridization conditions or alternatively, under lower stringency hybridization as defined *supra*. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X, or a fragment thereof), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or alternatively, under lower stringency hybridization conditions defined *supra*.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described *infra*. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, R. A., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985) further described in U.S. Patent No. 4,631,211.)

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, which specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes.

Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

Non-limiting examples of epitopes of polypeptides that can be used to generate antibodies of the invention include a polypeptide comprising, or alternatively consisting of, at least one, two, three, four, five, six or more of the portion(s) of SEQ ID NO:Y specified in Table 1B. These polypeptide fragments have been determined to bear antigenic epitopes of the proteins of the invention by the analysis of the Jameson-Wolf antigenic index, which is included in the DNASTar suite of computer programs. By "comprise" it is intended that a polypeptide contains at least one, two, three, four, five, six or more of the portion(s) of SEQ ID NO:Y shown in Table 1B, but it may contain additional flanking residues on either the amino or carboxyl termini of the recited portion. Such additional flanking sequences are preferably sequences naturally found adjacent to the portion; i.e., contiguous sequence shown in SEQ ID NO:Y. The flanking sequence may, however, be sequences from a heterologous polypeptide, such as from another protein described herein or from a heterologous polypeptide not described herein. In particular embodiments, epitope portions of a polypeptide of the invention comprise one, two, three, or more of the portions of SEQ ID NO:Y shown in Table 1B.

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, *in vivo* immunization, *in vitro* immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., J. Gen. Virol., 66:2347-2354 (1985). If *in vivo* immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides,

for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody that can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention (e.g., those comprising an immunogenic or antigenic epitope) can be fused to heterologous polypeptide sequences. For example, polypeptides of the present invention (including fragments or variants thereof), may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof, resulting in chimeric polypeptides. By way of another non-limiting example, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused with albumin (including but not limited to recombinant human serum albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). In a preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. Polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide). Polynucleotides encoding fusion proteins of the invention are also encompassed by the invention.

Such fusion proteins as those described above may facilitate purification and may increase half-life *in vivo*. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG fusion

proteins that have a disulfide-linked dimeric structure due to the IgG portion desulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin (HA) tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix-binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni²⁺ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Fusion Proteins

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, polypeptides of the present invention which are shown to be secreted can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

In certain preferred embodiments, proteins of the invention are fusion proteins comprising an amino acid sequence that is an N and/or C- terminal deletion of a polypeptide of the invention. In preferred embodiments, the invention is directed to a fusion protein comprising an amino acid sequence that is at least 90%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence of the invention. Polynucleotides encoding these proteins are also encompassed by the invention.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides is familiar and routine techniques in the art.

As one of skill in the art will appreciate that, as discussed above, polypeptides of the

present invention, and epitope-bearing fragments thereof, can be combined with heterologous polypeptide sequences. For example, the polypeptides of the present invention may be fused with heterologous polypeptide sequences, for example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), or albumin (including, but not limited to, native or recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties (EP-A 0232 262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., *J. Molecular Recognition* 8:52-58 (1995); K. Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995).

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a polypeptide that facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., *Cell* 37:767 (1984)).

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., *Curr. Opin. Biotechnol.* 8:724-33 (1997); Harayama, *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, et al., *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo and Blasco, *Biotechniques* 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by

these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

Recombinant and Synthetic Production of Polypeptides of the Invention

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by synthetic and recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides of the invention may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome-binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418, glutamine synthase, or neomycin resistance for eukaryotic cell culture, and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells (e.g., Saccharomyces cerevisiae or Pichia pastoris (ATCC Accession No.

201178)); insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulfoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors is the availability of cell lines (e.g., the murine myeloma cell line, NS0) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g., Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657, which are hereby incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors can be obtained from Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington *et al.*, *Bio/technology* 10:169(1992) and in Biblia and Robinson *Biotechnol. Prog.* 11:1 (1995), which are herein incorporated by reference.

The present invention also relates to host cells containing the above-described vector constructs described herein, and additionally encompasses host cells containing nucleotide sequences of the invention that are operably associated with one or more heterologous control regions (e.g., promoter and/or enhancer) using techniques known of in the art. The host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. A host strain may be chosen which modulates the expression of the inserted gene sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and

modification (e.g., phosphorylation, cleavage) of proteins. Appropriate cell lines can be chosen to ensure the desired modifications and processing of the foreign protein expressed.

Introduction of the nucleic acids and nucleic acid constructs of the invention into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., *Basic Methods In Molecular Biology* (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., the coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination (see, e.g., US Patent Number 5,641,670, issued June 24, 1997; International Publication Number WO 96/29411; International Publication Number WO 94/12650; Koller *et al.*, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); and Zijlstra *et al.*, *Nature* 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

Polypeptides of the invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins,

this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

5 In one embodiment, the yeast *Pichia pastoris* is used to express polypeptides of the invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O₂. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O₂. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See Ellis, S.B., *et al.*, *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., *et al.*, *Yeast* 5:167-77 (1989); Tschopp, J.F., *et al.*, *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

20 In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a polypeptide of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

25 Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

30 In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein,

the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with polynucleotides of the invention, and
5 which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4,
10 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a
15 polypeptide corresponding to a fragment of a polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-
20 diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino
25 acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides of the present invention which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an
30 antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for
35 example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine

residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

5 Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include
10 umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and
15 examples of suitable radioactive material include iodine (^{121}I , ^{123}I , ^{125}I , ^{131}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{111}In , ^{112}In , $^{113\text{m}}\text{In}$, $^{115\text{m}}\text{In}$), technetium (^{99}Tc , $^{99\text{m}}\text{Tc}$), thallium (^{201}Tl), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , and ^{97}Ru .

15 In specific embodiments, a polypeptide of the present invention or fragment or variant thereof is attached to macrocyclic chelators that associate with radiometal ions, including but not limited to, ^{177}Lu , ^{90}Y , ^{166}Ho , and ^{153}Sm , to polypeptides. In a preferred embodiment, the radiometal ion associated with the macrocyclic chelators is ^{111}In . In another preferred
20 embodiment, the radiometal ion associated with the macrocyclic chelator is ^{90}Y . In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane- $\text{N},\text{N}',\text{N}'',\text{N}'''$ -tetraacetic acid (DOTA). In other specific embodiments, DOTA is attached to an antibody of the invention or
25 fragment thereof via a linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., Clin Cancer Res. 4(10):2483-90 (1998); Peterson et al., Bioconjug. Chem. 10(4):553-7 (1999); and Zimmerman et al, Nucl. Med. Biol. 26(8):943-50 (1999); which are hereby incorporated by
reference in their entirety.

As mentioned, the proteins of the invention may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Polypeptides of the invention may
30 be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of
35 a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor

formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR
5 PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth. Enzymol. 182:626-646 (1990); Rattan et al., Ann. N.Y. Acad. Sci. 663:48-62 (1992)).

Also provided by the invention are chemically modified derivatives of the polypeptides of
10 the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions
15 within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will
20 weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about
25 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

30 As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

35 The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, such as, for example, the

method disclosed in EP 0 401 384 (coupling PEG to G-CSF), herein incorporated by reference; see also Malik et al., *Exp. Hematol.* 20:1028-1035 (1992), reporting pegylation of GM-CSF using tresyl chloride. For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation, which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado et al., *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis et al., *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ($\text{ClSO}_2\text{CH}_2\text{CF}_3$). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number of additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in International Publication No. WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

The number of polyethylene glycol moieties attached to each protein of the invention (i.e., the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado et al., *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

The polypeptides of the invention can be recovered and purified from chemical synthesis and recombinant cell cultures by standard methods which include, but are not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Well-known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and/or purification.

The polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and

multimers of the polypeptides of the invention, their preparation, and compositions (preferably, Therapeutics) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

5 Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer refers to a multimer containing only polypeptides corresponding to a protein of the invention (e.g., the amino acid sequence of SEQ ID NO:Y, an amino acid sequence encoded by SEQ ID NO:X or the complement of SEQ ID NO:X, the amino acid sequence encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, and/or an amino acid
10 sequence encoded by cDNA contained in ATCC Deposit No:Z (including fragments, variants, splice variants, and fusion proteins, corresponding to these as described herein)). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a
15 multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing two polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing three polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least
20 a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the
25 invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked by, for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In
30 another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such
35 covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in SEQ ID NO:Y, encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, and/or encoded by the cDNA contained in ATCC Deposit No:Z).

In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences that interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide

sequence. In a further embodiment, proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C-terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of the

invention (e.g., a polypeptide or fragment or variant of the amino acid sequence of SEQ ID NO:Y or a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z, and/or an epitope, of the present invention) as determined by immunoassays well known in the art for assaying specific antibody-antigen binding. Antibodies of the invention include, but are not limited to, polyclonal, 5 monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), intracellularly-made antibodies (i.e., intrabodies), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active 10 portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In preferred embodiments, the immunoglobulin molecules of the invention are IgG1. In other preferred embodiments, the immunoglobulin 15 molecules of the invention are IgG4.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may 20 comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse 25 and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

30 The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; 35 Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the

epitope(s) or portion(s) of a polypeptide of the present invention that they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, or by size in contiguous amino acid residues, or listed in the Tables and Figures. Preferred epitopes of the invention include the predicted epitopes shown in Table 1B, as well as polynucleotides that encode these epitopes. Antibodies that specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 5%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies that bind polypeptides encoded by polynucleotides that hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides

of the present invention. For example, the present invention includes antibodies that disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies that do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described *supra*). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies that bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies that bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies that activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, to purify, detect, and target the polypeptides of the present invention, including both *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies have utility in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor

Laboratory Press, 2nd ed. 1988); incorporated by reference herein in its entirety.

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalent and non-covalent conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387; the disclosures of which are incorporated herein by reference in their entireties.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is

not limited to antibodies produced through hybridoma technology. The term “monoclonal antibody” refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples. In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Another well-known method for producing both polyclonal and monoclonal human B cell lines is transformation using Epstein Barr Virus (EBV). Protocols for generating EBV-transformed B cell lines are commonly known in the art, such as, for example, the protocol outlined in Chapter 7.22 of *Current Protocols in Immunology*, Coligan et al., Eds., 1994, John Wiley & Sons, NY, which is hereby incorporated in its entirety by reference. The source of B cells for transformation is commonly human peripheral blood, but B cells for transformation may also be derived from other sources including, but not limited to, lymph nodes, tonsil, spleen, tumor tissue, and infected tissues. Tissues are generally made into single cell suspensions prior to EBV transformation. Additionally, steps may be taken to either physically remove or inactivate T cells (e.g., by treatment with cyclosporin A) in B cell-containing samples, because T cells from individuals seropositive for anti-EBV antibodies can suppress B cell immortalization by EBV.

In general, the sample containing human B cells is inoculated with EBV, and cultured for 3-4 weeks. A typical source of EBV is the culture supernatant of the B95-8 cell line (ATCC #VR-1492). Physical signs of EBV transformation can generally be seen towards the end of the 3-4 week culture period. By phase-contrast microscopy, transformed cells may appear large, clear, hairy and tend to aggregate in tight clusters of cells. Initially, EBV lines are generally polyclonal. However, over prolonged periods of cell cultures, EBV lines may become monoclonal or

polyclonal as a result of the selective outgrowth of particular B cell clones. Alternatively, polyclonal EBV transformed lines may be subcloned (e.g., by limiting dilution culture) or fused with a suitable fusion partner and plated at limiting dilution to obtain monoclonal B cell lines. Suitable fusion partners for EBV transformed cell lines include mouse myeloma cell lines (e.g., SP2/0, X63-Ag8.653), heteromyeloma cell lines (human x mouse; e.g., SPAM-8, SBC-H20, and CB-F7), and human cell lines (e.g., GM 1500, SKO-007, RPMI 8226, and KR-4). Thus, the present invention also provides a method of generating polyclonal or monoclonal human antibodies against polypeptides of the invention or fragments thereof, comprising EBV-transformation of human B cells.

Antibody fragments that recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')₂ fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles that carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187 9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below.

For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring that express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; 5,939,598; 6,075,181; and 6,114,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies that recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444; (1989) and Nissinoff, J. *Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization

and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand(s)/receptor(s). For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its
5 ligand(s)/receptor(s), and thereby block its biological activity. Alternatively, antibodies which bind to and enhance polypeptide multimerization and/or binding, and/or receptor/ligand multimerization, binding and/or signaling can be used to generate anti-idiotypes that function as agonists of a polypeptide of the invention and/or its ligand/receptor. Such agonistic anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens as agonists of the
10 polypeptides of the invention or its ligand(s)/receptor(s). For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligand(s)/receptor(s), and thereby promote or enhance its biological activity.

Intrabodies of the invention can be produced using methods known in the art, such as those disclosed and reviewed in Chen et al., Hum. Gene Ther. 5:595-601 (1994); Marasco, W.A.,
15 Gene Ther. 4:11-15 (1997); Rondon and Marasco, Annu. Rev. Microbiol. 51:257-283 (1997); Proba et al., J. Mol. Biol. 275:245-253 (1998); Cohen et al., Oncogene 17:2445-2456 (1998); Ohage and Steipe, J. Mol. Biol. 291:1119-1128 (1999); Ohage et al., J. Mol. Biol. 291:1129-1134 (1999); Wirtz and Steipe, Protein Sci. 8:2245-2250 (1999); Zhu et al., J. Immunol. Methods 231:207-222 (1999); and references cited therein.

Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or alternatively, under lower stringency
25 hybridization conditions, e.g., as defined *supra*, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y, to a polypeptide encoded by a portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, and/or to a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically
30 synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then
35 amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid

from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described *supra*. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed *supra*, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et

al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described *supra*, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques. Methods of producing antibodies include, but are not limited to, hybridoma technology, EBV transformation, and other methods discussed herein as well as through the use recombinant DNA technology, as discussed below.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods that are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO

86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

5 The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be
10 co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which
15 may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing
20 antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293,
25 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule.
30 For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected
35 depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion

protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and

processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgp^rt- or ap^rt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215 (1993)); and hyg^r, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994);

Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulfoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors are the availability of cell lines (e.g., the murine myeloma cell line, NS0) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g. Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657 which are incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors that may be used according to the present invention are commercially available from suppliers, including, for example Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington *et al.*, *Bio/technology* 10:169(1992) and in Biblia and Robinson *Biotechnol. Prog.* 11:1 (1995) which are incorporated in their entireties by reference herein.

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature* 322:52 (1986); Kohler, *Proc. Natl. Acad. Sci. USA* 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the

purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or *in vivo*, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., *supra*, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452 (1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337-11341 (1992) (said references incorporated by reference in their entireties).

As discussed, *supra*, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the *in vivo* half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or

conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See EP 394,827; and Traunecker et al., *Nature* 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide-linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. See, for example, Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. See, for example, EP A 232,262. Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., *J. Molecular Recognition* 8:52-58 (1995); Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995)).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., *Cell* 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions that can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase,

alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ¹²⁵I, ¹³¹I, ¹¹¹In or ⁹⁹Tc.

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ²¹³Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGF (See, International Publication No. WO 99/23105), a thrombotic agent or an anti- angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or

polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known. See, for example, Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. Translation products of the gene of the present invention may be useful as cell-specific markers, or more specifically as cellular markers that are differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al., eds., (1994), Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, section 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ³²P or ¹²⁵I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g.,

Ausubel et al, eds, (1994), Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, section 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, (1994), Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, section 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., 3H or 125I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., 3H or 125I) in the presence of increasing amounts of an unlabeled second antibody.

Antibodies of the invention may be characterized using immunocytochemistry methods on cells (e.g., mammalian cells, such as CHO cells) transfected with a vector enabling the expression of an antigen or with vector alone using techniques commonly known in the art. Antibodies that bind antigen transfected cells, but not vector-only transfected cells, are antigen specific.

Therapeutic Uses

Table 1D also provides information regarding biological activities and preferred therapeutic uses (i.e. see, "Preferred Indications" column) for polynucleotides and polypeptides of the invention (including antibodies, agonists, and/or antagonists thereof). Table 1D also provides information regarding assays which may be used to test polynucleotides and polypeptides of the invention (including antibodies, agonists, and/or antagonists thereof) for the corresponding biological activities. The first column ("Gene No.") provides the gene number in the application for each clone identifier. The second column ("cDNA ATCC Deposit No:Z") provides the unique

clone identifier for each clone as previously described and indicated in Table 1A, Table 1B, and Table 1C. The third column ("AA SEQ ID NO:Y") indicates the Sequence Listing SEQ ID Number for polypeptide sequences encoded by the corresponding cDNA clones (also as indicated in Table 1A, Table 1B, and Table 2). The fourth column ("Biological Activity") indicates a biological activity corresponding to the indicated polypeptides (or polynucleotides encoding said polypeptides). The fifth column ("Exemplary Activity Assay") further describes the corresponding biological activity and also provides information pertaining to the various types of assays that may be performed to test, demonstrate, or quantify the corresponding biological activity.

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to detect, prevent, diagnose, prognosticate, treat, and/or ameliorate diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, allergic and/or asthmatic diseases and disorders. The treatment and/or prevention of allergic and/or asthmatic diseases and disorders associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with allergic and/or asthmatic diseases and disorders. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

In a specific and preferred embodiment, the present invention is directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating allergic and/or asthmatic diseases and disorders. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (e.g., antibodies directed to the full length protein expressed on the cell surface of a mammalian cell; antibodies directed to an epitope of a polypeptide of the invention (such as, for example, a predicted linear epitope shown in Table 1B; or a conformational epitope, including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to detect, diagnose, prevent, treat, prognosticate, and/or ameliorate allergic and/or asthmatic diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention. The treatment and/or prevention of allergic and/or asthmatic diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but

is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

5 A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without
10 undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells that interact with the antibodies.

15 The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.
20

It is preferred to use high affinity and/or potent *in vivo* inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of allergic and/or asthmatic diseases or disorders related to polynucleotides or polypeptides, including fragments thereof, of the present
25 invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.
30

Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent an allergic and/or
35 asthmatic disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the

invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical
5 Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev.
Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and
Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993).
Methods commonly known in the art of recombinant DNA technology which can be used are
described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons,
10 NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press,
NY (1990).

In a preferred embodiment, the compound comprises nucleic acid sequences encoding an
antibody, said nucleic acid sequences being part of expression vectors that express the antibody or
fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular,
15 such nucleic acid sequences have promoters operably linked to the antibody coding region, said
promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular
embodiment, nucleic acid molecules are used in which the antibody coding sequences and any
other desired sequences are flanked by regions that promote homologous recombination at a
desired site in the genome, thus providing for intrachromosomal expression of the antibody
20 encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989);
Zijlstra et al., Nature 342:435-438 (1989). In specific embodiments, the expressed antibody
molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences
encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient
25 is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case,
cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These
two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*,
where it is expressed to produce the encoded product. This can be accomplished by any of
30 numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic
acid expression vector and administering it so that they become intracellular, e.g., by infection
using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or
by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun;
Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents,
35 encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage
to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject
to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987))

(which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contain nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitate delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143-155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral

infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

5 In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler
10 and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible
15 by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

20 Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as
25 obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic
30 effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

35 In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by the presence or absence of an appropriate inducer of transcription.

Demonstration of Therapeutic or Prophylactic Activity

The compounds or pharmaceutical compositions of the invention are preferably tested *in vitro*, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans.

5 For example, *in vitro* assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, *in vitro* assays
10 which can be used to determine whether administration of a specific compound is indicated, include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

15 *Therapeutic/Prophylactic Administration and Composition*

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably a polypeptide or antibody of the invention. In a preferred embodiment, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce
20 undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate
25 formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J.
30 Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and
35 intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by

any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

5 In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous,
10 non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

 In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the*
15 *Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

 In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351
20 (1989); Howard et al., *J.Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, e.g., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)).

 Other controlled release systems are discussed in the review by Langer (*Science*
30 249:1527-1533 (1990)).

 In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent
35 No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see

e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

5 The present invention also provides pharmaceutical compositions. Such compositions
comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable
carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a
regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other
generally recognized pharmacopeia for use in animals, and more particularly in humans. The term
"carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is
10 administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including
those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral
oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is
administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also
be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical
15 excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel,
sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol,
propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor
amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take
the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release
20 formulations and the like. The composition can be formulated as a suppository, with traditional
binders and carriers such as triglycerides. Oral formulation can include standard carriers such as
pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine,
cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described
in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a
25 therapeutically effective amount of the compound, preferably in purified form, together with a
suitable amount of carrier so as to provide the form for proper administration to the patient. The
formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine
procedures as a pharmaceutical composition adapted for intravenous administration to human
30 beings. Typically, compositions for intravenous administration are solutions in sterile isotonic
aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a
local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the
ingredients are supplied either separately or mixed together in unit dosage form, for example, as a
dry lyophilized powder or water free concentrate in a hermetically sealed container such as an
35 ampoule or sachette indicating the quantity of active agent. Where the composition is to be
administered by infusion, it can be dispensed with an infusion bottle containing sterile
pharmaceutical grade water or saline. Where the composition is administered by injection, an

ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Diagnosis and Imaging

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, prognosticate, or monitor allergic and/or asthmatic diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the

expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

5 The invention provides a diagnostic assay for diagnosing an allergic and/or asthmatic disease or disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard
10 expression level is indicative of a particular allergic and/or asthmatic disease or disorder. With respect to an allergic and/or asthmatic disease or disorder, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health
15 professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the allergic and/or asthmatic disease or disorder.

 Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen et al., J. Cell. Biol. 105:3087-3096 (1987)). Other
20 antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (¹²⁵I, ¹²¹I), carbon (¹⁴C), sulfur (³⁵S), tritium (³H), indium (¹¹²In), and technetium (⁹⁹Tc); luminescent labels, such as luminol; and fluorescent labels, such
25 as fluorescein and rhodamine, and biotin.

 One facet of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled
30 molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level
35 indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for

a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells that contain the specific protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disorder, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope that is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control

antibody that does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

5 In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope that is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

10 In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

20 In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

30 In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme that is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome, thus each polynucleotide of the present invention can routinely be used as a chromosome marker using techniques known in the art. Table 1B.1, column 8 provides the chromosome location of some of the polynucleotides of the invention.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably at least 15 bp (e.g., 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can optionally be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, preselection by hybridization to construct chromosome specific-cDNA libraries, and computer mapping techniques (See, e.g., Shuler, Trends Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety).

Precise chromosomal location of the polynucleotides can also be achieved using

fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

5 For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes).

Thus, the present invention also provides a method for chromosomal localization which involves (a) preparing PCR primers from the polynucleotide sequences in Table 1B and/or Table 2
10 and SEQ ID NO:X and (b) screening somatic cell hybrids containing individual chromosomes.

The polynucleotides of the present invention would likewise be useful for radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g. Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London (1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al.,
15 Mol. Psychiatry 3:483-492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000); and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes
20 coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library)). Column 9 of Table 1B.1 provides an OMIM reference identification number of diseases associated with the cytologic band disclosed in column 8 of Table 1B.1, as determined using techniques described herein and by
25 reference to Table 5. Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in a polynucleotide of the invention and the corresponding gene between affected and unaffected individuals can be examined. First,
30 visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is
35 required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as

compared to unaffected individuals can be assessed using the polynucleotides of the invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker. Diagnostic and prognostic methods, kits and reagents encompassed by the present invention are briefly described below and more thoroughly elsewhere herein (see e.g., the sections labeled "Antibodies", "Diagnostic Assays", and "Methods for
5 Detecting Diseases").

Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an individual and comparing the measured gene expression level with a
10 standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder. Additional non-limiting examples of diagnostic methods encompassed by the present invention are more thoroughly described elsewhere herein (see, e.g., Example 12).

In still another embodiment, the invention includes a kit for analyzing samples for the
15 presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the invention, where each probe has one strand containing a 31'-mer-end internal
20 to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a related disorder, including, for example, diagnosis of a tumor, has already been made according to conventional methods, the present invention is useful as a
25 prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotides of the invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the invention or the level of the mRNA encoding the polypeptide of the invention in a first biological sample
30 either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the related
35 disorder or being determined by averaging levels from a population of individuals not having a related disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source that contains polypeptide of the present invention or the corresponding mRNA. As indicated, biological samples include body fluids (such as semen, lymph, vaginal pool, sera, plasma, urine, synovial fluid and spinal fluid) that contain the polypeptide of the present invention, and tissue sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides of the invention are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the invention attached may be used to identify polymorphisms between the isolated polynucleotide sequences of the invention, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, such as for example, in neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, digestive disorders, metabolic disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced *supra* are hereby incorporated by reference in their entirety herein.

The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides of the invention are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by Nielsen et al., Science 254, 1497 (1991); and Egholm et al., Nature 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a

PNA/DNA 15-mer lowers the melting point ($T_{sub.m}$) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

The compounds of the present invention have uses that include, but are not limited to, detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative disorders are often associated with inappropriate activation of proto-oncogenes. (Germann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in Neoplastic Diseases of the Blood, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Germann et al., *supra*) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Germann et al., *supra*) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Germann et al., *supra*)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580). However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness is not be limited to treatment, prevention, and/or prognosis of proliferative disorders of cells and tissues of hematopoietic origin, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

In addition to the foregoing, a polynucleotide of the present invention can be used to control gene expression through triple helix formation or through antisense DNA or RNA.

Antisense techniques are discussed, for example, in Okano, J. *Neurochem.* 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., *Nucleic Acids Research* 6: 3073 (1979); Cooney et al., *Science* 241: 456 (1988); and Dervan et al., *Science* 251: 1360 (1991).

5 Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., *Nucl. Acids Res.* 6:3073 (1979); Cooney et al., *Science* 241:456 (1988); and Dervan et al., *Science* 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. *Neurochem.* 56:560 (1991);
10 Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. The oligonucleotide described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of polypeptide of the present invention
15 antigens. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease, and in particular, for the treatment of proliferative diseases and/or conditions. Non-limiting antisense and triple helix methods encompassed by the present invention are more thoroughly described elsewhere herein (see, e.g., the section labeled "Antisense and Ribozyme (Antagonists)").

20 Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell. Additional
25 non-limiting examples of gene therapy methods encompassed by the present invention are more thoroughly described elsewhere herein (see, e.g., the sections labeled "Gene Therapy Methods", and Examples 16, 17 and 18).

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment
30 length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for
35 RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's

genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR Technology, Freeman and Co. (1992)). Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers prepared from the sequences of the present invention, specific to tissues, including but not limited to those shown in Table 1B. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination. Additional non-limiting examples of such uses are further described herein.

The polynucleotides of the present invention are also useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays) or cell type(s) (e.g., immunocytochemistry assays). In addition, for a number of disorders of the above tissues or cells, significantly higher or lower levels of gene expression of the polynucleotides/polypeptides of the present invention may be detected in certain tissues (e.g., tissues expressing polypeptides and/or polynucleotides of the present invention, for example, those disclosed in column 7 of Table 1B.1, and/or cancerous and/or wounded tissues) or bodily fluids (e.g., semen, lymph, vaginal pool, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

Thus, the invention provides a diagnostic method of a disorder, which involves: (a) assaying gene expression level in cells or body fluid of an individual; (b) comparing the gene expression level with a standard gene expression level, whereby an increase or decrease in the

assayed gene expression level compared to the standard expression level is indicative of a disorder.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

10 Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

Polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays such as, for example, ABC immunoperoxidase (Hsu et al., J. Histochem. Cytochem. 29:577-580 (1981)) or cell type(s) (e.g., immunocytochemistry assays).

Antibodies can be used to assay levels of polypeptides encoded by polynucleotides of the invention in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{131}I , ^{125}I , ^{123}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium ($^{115\text{m}}\text{In}$, $^{113\text{m}}\text{In}$, ^{112}In , ^{111}In), and technetium (^{99}Tc , $^{99\text{m}}\text{Tc}$), thallium (^{201}Tl), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru ; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying levels of polypeptide of the present invention in a biological sample, proteins can also be detected *in vivo* by imaging. Antibody labels or markers for *in vivo* imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I , ^{112}In , $^{99\text{m}}\text{Tc}$, ^{131}I ,

¹²⁵I, ¹²³I, ¹²¹I), carbon (¹⁴C), sulfur (³⁵S), tritium (³H), indium (^{115m}In, ^{113m}In, ¹¹²In, ¹¹¹In), and technetium (⁹⁹Tc, ^{99m}Tc), thallium (²⁰¹Tl), gallium (⁶⁸Ga, ⁶⁷Ga), palladium (¹⁰³Pd), molybdenum (⁹⁹Mo), xenon (¹³³Xe), fluorine (¹⁸F, ¹⁵³Sm, ¹⁷⁷Lu, ¹⁵⁹Gd, ¹⁴⁹Pm, ¹⁴⁰La, ¹⁷⁵Yb, ¹⁶⁶Ho, ⁹⁰Y, ⁴⁷Sc, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁴²Pr, ¹⁰⁵Rh, ⁹⁷Ru), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells that express the polypeptide encoded by a polynucleotide of the invention. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (e.g., polypeptides encoded by polynucleotides of the invention and/or antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention in association with toxins or cytotoxic prodrugs.

By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ²¹³Bi, or other radioisotopes such as, for example, ¹⁰³Pd, ¹³³Xe, ¹³¹I, ⁶⁸Ge, ⁵⁷Co, ⁶⁵Zn, ⁸⁵Sr, ³²P, ³⁵S, ⁹⁰Y, ¹⁵³Sm, ¹⁵³Gd, ¹⁶⁹Yb, ⁵¹Cr, ⁵⁴Mn, ⁷⁵Se, ¹¹³Sn, ⁹⁰Yttrium, ¹¹⁷Tin, ¹⁸⁶Rhenium,

¹⁶⁶Holmium, and ¹⁸⁸Rhenium; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin. In a specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the radioisotope ⁹⁰Y. In another specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the radioisotope ¹¹¹In. In a further specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the radioisotope ¹³¹I.

Techniques known in the art may be applied to label polypeptides of the invention (including antibodies). Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression level of a polypeptide of the present invention in cells or body fluid of an individual; and (b) comparing the assayed polypeptide expression level with a standard polypeptide expression level, whereby an increase or decrease in the assayed polypeptide expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, polypeptides of the present invention can be used to treat or prevent diseases or conditions such as, for example, neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition,

enhancement of the immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease (as described *supra*, and elsewhere herein). For example, administration of an antibody directed to a polypeptide of the present invention can bind, and/or neutralize the polypeptide, and/or reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the biological activities described herein.

Diagnostic Assays

The compounds of the present invention are useful for diagnosis, treatment, prevention and/or prognosis of various disorders in mammals, preferably humans. Such disorders include, but are not limited to, those related to biological activities described in Table 1D and, also as described herein under the section heading "Biological Activities".

For a number of disorders, substantially altered (increased or decreased) levels of gene expression can be detected in tissues, cells or bodily fluids (e.g., sera, plasma, urine, semen, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" gene expression level, that is, the expression level in tissues or bodily fluids from an individual not having the disorder. Thus, the invention provides a diagnostic method useful during diagnosis of a disorder, which involves measuring the expression level of the gene encoding the polypeptide in tissues, cells or body fluid from an individual and comparing the measured gene expression level with a standard gene expression level, whereby an increase or decrease in the gene expression level(s) compared to the standard is indicative of a disorder. These diagnostic assays may be performed *in vivo* or *in vitro*, such as, for example, on blood samples, biopsy tissue or autopsy tissue.

The present invention is also useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed gene expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

In certain embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to diagnose and/or prognosticate diseases and/or disorders associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table

1B.2, column 5 (Tissue Distribution Library Code).

By "assaying the expression level of the gene encoding the polypeptide" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the invention or the level of the mRNA encoding the polypeptide of the invention in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or
5 relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide expression level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the
10 disorder or being determined by averaging levels from a population of individuals not having the disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source containing polypeptides of the invention (including portions
15 thereof) or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) and tissue sources found to express the full length or fragments thereof of a polypeptide or mRNA. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

Total cellular RNA can be isolated from a biological sample using any suitable technique
20 such as the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, *Anal. Biochem.* 162:156-159 (1987). Levels of mRNA encoding the polypeptides of the invention are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse
25 transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of polypeptides of the invention, in a biological sample (e.g., cells and tissues), including determination of normal and abnormal levels of polypeptides. Thus, for
30 instance, a diagnostic assay in accordance with the invention for detecting over-expression of polypeptides of the invention compared to normal control tissue samples may be used to detect the presence of tumors. Assay techniques that can be used to determine levels of a polypeptide, such as a polypeptide of the present invention in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays,
35 Western Blot analysis and ELISA assays. Assaying polypeptide levels in a biological sample can occur using any art-known method.

Assaying polypeptide levels in a biological sample can occur using antibody-based

techniques. For example, polypeptide expression in tissues can be studied with classical immunohistological methods (Jalkanen et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting polypeptide gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{112}In), and technetium ($^{99\text{m}}\text{Tc}$), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

The tissue or cell type to be analyzed will generally include those that are known, or suspected, to express the gene of interest (such as, for example, cancer). The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by reference in its entirety. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells that could be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the gene.

For example, antibodies, or fragments of antibodies, such as those described herein, may be used to quantitatively or qualitatively detect the presence of gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

In a preferred embodiment, antibodies, or fragments of antibodies directed to any one or all of the predicted epitope domains of the polypeptides of the invention (shown in Table 1B) may be used to quantitatively or qualitatively detect the presence of gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

In an additional preferred embodiment, antibodies, or fragments of antibodies directed to a conformational epitope of a polypeptide of the invention may be used to quantitatively or qualitatively detect the presence of gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

The antibodies (or fragments thereof), and/or polypeptides of the present invention may, additionally, be employed histologically, as in immunofluorescence, immunoelectron microscopy or non-immunological assays, for in situ detection of gene products or conserved variants or

peptide fragments thereof. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody or polypeptide of the present invention. The antibody (or fragment thereof) or polypeptide is preferably applied by overlaying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the gene product, or conserved variants or peptide fragments, or polypeptide binding, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

Immunoassays and non-immunoassays for gene products or conserved variants or peptide fragments thereof will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectably labeled antibody capable of binding gene products or conserved variants or peptide fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support that is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled antibody or detectable polypeptide of the invention. The solid phase support may then be washed with the buffer a second time to remove unbound antibody or polypeptide. Optionally the antibody is subsequently labeled. The amount of bound label on solid support may then be detected by conventional means.

By "solid phase support or carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

The binding activity of a given lot of antibody or antigen polypeptide may be determined according to well-known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

In addition to assaying polypeptide levels or polynucleotide levels in a biological sample

obtained from an individual, polypeptide or polynucleotide can also be detected *in vivo* by imaging. For example, in one embodiment of the invention, polypeptides and/or antibodies of the invention are used to image diseased cells, such as neoplasms. In another embodiment, polynucleotides of the invention (e.g., polynucleotides complementary to all or a portion of an mRNA) and/or antibodies (e.g., antibodies directed to any one or a combination of the epitopes of a polypeptide of the invention, antibodies directed to a conformational epitope of a polypeptide of the invention, or antibodies directed to the full length polypeptide expressed on the cell surface of a mammalian cell) are used to image diseased or neoplastic cells.

Antibody labels or markers for *in vivo* imaging of polypeptides of the invention include those detectable by X-radiography, NMR, MRI, CAT-scans or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. Where *in vivo* imaging is used to detect enhanced levels of polypeptides for diagnosis in humans, it may be preferable to use human antibodies or "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using techniques described herein or otherwise known in the art. For example methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., *Nature* 312:643 (1984); Neuberger et al., *Nature* 314:268 (1985).

Additionally, any polypeptides of the invention whose presence can be detected can be administered. For example, polypeptides of the invention labeled with a radio-opaque or other appropriate compound can be administered and visualized *in vivo*, as discussed, above for labeled antibodies. Further, such polypeptides can be utilized for *in vitro* diagnostic procedures.

A polypeptide-specific antibody or antibody fragment that has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I , ^{112}In , $^{99\text{m}}\text{Tc}$), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for a disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of $^{99\text{m}}\text{Tc}$. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells that contain the antigenic protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

With respect to antibodies, one of the ways in which an antibody of the present invention can be detectably labeled is by linking the same to a reporter enzyme and using the linked product in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller et al., *J. Clin. Pathol.* 31:507-520 (1978); Butler, J.E., *Meth. Enzymol.* 73:482-523 (1981); Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL.; Ishikawa, E. et al., (eds.), 1981, Enzyme Immunoassay, Kaku Shoin, Tokyo). The reporter enzyme that is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Reporter enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. Additionally, the detection can be accomplished by colorimetric methods that employ a chromogenic substrate for the reporter enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect polypeptides through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by means including, but not limited to, a gamma counter, a scintillation counter, or autoradiography.

It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, ophthaldehyde and fluorescamine.

The antibody can also be detectably labeled using fluorescence emitting metals such as ¹⁵²Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction.

Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt, and oxalate ester.

Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which
5 a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

Methods for Detecting Diseases

10 In general, a disease may be detected in a patient based on the presence of one or more proteins of the invention and/or polynucleotides encoding such proteins in a biological sample (for example, blood, sera, urine, and/or tumor biopsies) obtained from the patient. In other words, such proteins may be used as markers to indicate the presence or absence of a disease or disorder, including cancer and/or as described elsewhere herein. In addition, such proteins may be useful for
15 the detection of other diseases and cancers. The binding agents provided herein generally permit detection of the level of antigen that binds to the agent in the biological sample. Polynucleotide primers and probes may be used to detect the level of mRNA encoding polypeptides of the invention, which is also indicative of the presence or absence of a disease or disorder, including cancer. In general, polypeptides of the invention should be present at a level that is at least three
20 fold higher in diseased tissue than in normal tissue.

There are a variety of assay formats known to those of ordinary skill in the art for using a binding agent to detect polypeptide markers in a sample. See, e.g., Harlow and Lane, *supra*. In general, the presence or absence of a disease in a patient may be determined by (a) contacting a biological sample obtained from a patient with a binding agent; (b) detecting in the sample a level
25 of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined cut-off value.

In a preferred embodiment, the assay involves the use of a binding agent(s) immobilized on a solid support to bind to and remove the polypeptide of the invention from the remainder of the sample. The bound polypeptide may then be detected using a detection reagent that contains a
30 reporter group and specifically binds to the binding agent/polypeptide complex. Such detection reagents may comprise, for example, a binding agent that specifically binds to the polypeptide or an antibody or other agent that specifically binds to the binding agent, such as an anti-immunoglobulin, protein G, protein A or a lectin. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the
35 immobilized binding agent after incubation of the binding agent with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the binding agent is indicative of the reactivity of the sample with the immobilized binding agent. Suitable

polypeptides for use within such assays include polypeptides of the invention and portions thereof, or antibodies, to which the binding agent binds, as described above.

The solid support may be any material known to those of skill in the art to which polypeptides of the invention may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The binding agent may be immobilized on the solid support using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the agent and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the binding agent, in a suitable buffer, with the solid support for the suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and about 1 day. In general, contacting a well of plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about 10 ug, and preferably about 100 ng to about 1 ug, is sufficient to immobilize an adequate amount of binding agent.

Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (see, e.g., Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

Gene Therapy Methods

Also encompassed by the invention are gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of the polypeptide of the present invention. This method requires a polynucleotide that codes for a polypeptide of the present invention operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein

incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the present invention *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide of the present invention. Such methods are well-known in the art. For example, see Belldgrun, A., et al., J. Natl. Cancer Inst. 85: 207-216 (1993); Ferrantini, M. et al., Cancer Research 53: 1107-1112 (1993); Ferrantini, M. et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura, H., et al., Cancer Research 50: 5102-5106 (1990); Santodonato, L., et al., Human Gene Therapy 7:1-10 (1996); Santodonato, L., et al., Gene Therapy 4:1246-1255 (1997); and Zhang, J.-F. et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells that are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the present invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotide of the present invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of the polynucleotide sequence. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes

Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotide of the present invention.

5 Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

10 The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is
15 similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells that are differentiated, although delivery and expression may be achieved in non-differentiated or less
20 completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably
25 from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

30 The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

35 The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA (1989) 86:6077-6081, which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem. (1990) 265:10189-10192, which is herein incorporated by reference), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication No. WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., P. Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC

under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., *Methods of Immunology* (1983), 101:512-527, which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include Ca^{2+} -EDTA chelation (Papahadjopoulos et al., *Biochim. Biophys. Acta* (1975) 394:483; Wilson et al., *Cell* 17:77 (1979)); ether injection (Deamer, D. and Bangham, A., *Biochim. Biophys. Acta* 443:629 (1976); Ostro et al., *Biochem. Biophys. Res. Commun.* 76:836 (1977); Fraley et al., *Proc. Natl. Acad. Sci. USA* 76:3348 (1979)); detergent dialysis (Enoch, H. and Strittmatter, P., *Proc. Natl. Acad. Sci. USA* 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., *J. Biol. Chem.* 255:10431 (1980); Szoka, F. and Papahadjopoulos, D., *Proc. Natl. Acad. Sci. USA* 75:145 (1978); Schaefer-Ridder et al., *Science* 215:166 (1982)), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent No. 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466,

5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 provides methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, ex vivo or *in vivo*, using a retroviral particle containing RNA that comprises a sequence encoding a polypeptide of the present invention.

5 Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

10 The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, 15 electroporation, the use of liposomes, and CaPO₄ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles that include polynucleotide encoding a polypeptide of the present invention. Such retroviral vector particles 20 then may be employed, to transduce eukaryotic cells, either in vitro or *in vivo*. The transduced eukaryotic cells will express a polypeptide of the present invention.

In certain other embodiments, cells are engineered, ex vivo or *in vivo*, with polynucleotide contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses a polypeptide of the present invention, and at the same time is inactivated in terms of its 25 ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz et al. Am. Rev. Respir. Dis.109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of 30 instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld, M. A. et al. (1991) Science 252:431-434; Rosenfeld et al., (1992) Cell 68:143-155). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) Proc. Natl. Acad. Sci. USA 76:6606).

Suitable adenoviral vectors useful in the present invention are described, for example, in 35 Kozarsky and Wilson, Curr. Opin. Genet. Devel. 3:499-503 (1993); Rosenfeld et al., Cell 68:143-155 (1992); Engelhardt et al., Human Genet. Ther. 4:759-769 (1993); Yang et al., Nature Genet. 7:362-369 (1994); Wilson et al., Nature 365:691-692 (1993); and U.S. Patent No. 5,652,224,

which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest that is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, *ex vivo* or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, N., Curr. Topics in Microbiol. Immunol. 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles that contain the polynucleotide construct. These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express a polypeptide of the invention.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding a polypeptide of the present invention) via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), which are herein

incorporated by reference. This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

The polynucleotide encoding a polypeptide of the present invention may contain a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral

or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers (Kaneda et al.,
5 Science 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably,
10 millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site. In specific embodiments, suitable delivery vehicles for use with systemic administration comprise liposomes comprising
15 polypeptides of the invention for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA 189:11277-11281, 1992, which is
25 incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the
30 skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as
35 the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian.

Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly preferred.

5 **Biological Activities**

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, can be used in assays to test for one or more biological activities. If these polynucleotides or polypeptides, or agonists or antagonists of the present invention, do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the
10 biological activity. Thus, the polynucleotides and polypeptides, and agonists or antagonists could be used to treat the associated disease.

Members of the secreted family of proteins are believed to be involved in biological activities associated with, for example, cellular signaling. Accordingly, compositions of the invention (including polynucleotides, polypeptides and antibodies of the invention, and fragments
15 and variants thereof) may be used in diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders associated with aberrant activity of secreted polypeptides.

In preferred embodiments, compositions of the invention (including polynucleotides, polypeptides and antibodies of the invention, and fragments and variants thereof) may be used in the diagnosis, prognosis, prevention, treatment, and/or amelioration of diseases and/or disorders
20 relating to the immune system (e.g., allergic reactions, asthma, anaphylaxis, hypersensitivity to an antigenic molecule, rhinitis, eczema, and as described in the "Gastrointestinal Disorders", "Respiratory Disorders", and "Wound Healing and Epithelial Cell Proliferation" sections below).

In certain embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to diagnose and/or
25 prognosticate diseases and/or disorders associated with the tissue(s) in which the polypeptide of the invention is expressed including one, two, three, four, five, or more tissues disclosed in Table 1B.2, column 5 (Tissue Distribution Library Code).

Thus, polynucleotides, translation products and antibodies of the invention are useful in the diagnosis, detection, prevention, prognostication, and/or treatment of diseases and/or disorders
30 associated with activities that include, but are not limited to, prohormone activation, neurotransmitter activity, cellular signaling, cellular proliferation, cellular differentiation, and cell migration.

More generally, polynucleotides, translation products and antibodies corresponding to this gene may be useful for the diagnosis, prognosis, prevention, treatment and/or amelioration of
35 diseases and/or disorders associated with the following system or systems.

Immune Activity

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in preventing, diagnosing, prognosticating, treating, and/or ameliorating diseases, disorders, and/or conditions of the immune system, by, for example, activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to treat diseases and disorders of the immune system and/or to inhibit or enhance an immune response generated by cells associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 1B.2, column 5 (Tissue Distribution Library Code).

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in preventing, diagnosing, prognosticating, treating and/or ameliorating immunodeficiencies, including both congenital and acquired immunodeficiencies. Examples of B cell immunodeficiencies in which immunoglobulin levels B cell function and/or B cell numbers are decreased include: X-linked agammaglobulinemia (Bruton's disease), X-linked infantile agammaglobulinemia, X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, X-linked lymphoproliferative syndrome (XLP), agammaglobulinemia including congenital and acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, unspecified hypogammaglobulinemia, recessive agammaglobulinemia (Swiss type), Selective IgM deficiency, selective IgA deficiency, selective IgG subclass deficiencies, IgG subclass deficiency (with or without IgA deficiency), Ig deficiency with increased IgM, IgG and IgA deficiency with increased IgM, antibody deficiency with normal or elevated Igs, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), common variable immunodeficiency (CVID), common variable immunodeficiency (CVI) (acquired), and transient hypogammaglobulinemia of infancy.

In specific embodiments, ataxia-telangiectasia or conditions associated with ataxia-telangiectasia are detected, prevented, diagnosed, prognosticated, treated, and/or ameliorated using

the polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof.

Examples of congenital immunodeficiencies in which T cell and/or B cell function and/or number is decreased include, but are not limited to: DiGeorge anomaly, severe combined immunodeficiencies (SCID) (including, but not limited to, X-linked SCID, autosomal recessive SCID, adenosine deaminase deficiency, purine nucleoside phosphorylase (PNP) deficiency, Class II MHC deficiency (Bare lymphocyte syndrome), Wiskott-Aldrich syndrome, and ataxia telangiectasia), thymic hypoplasia, third and fourth pharyngeal pouch syndrome, 22q11.2 deletion, chronic mucocutaneous candidiasis, natural killer cell deficiency (NK), idiopathic CD4+ T-lymphocytopenia, immunodeficiency with predominant T cell defect (unspecified), and unspecified immunodeficiency of cell mediated immunity.

In specific embodiments, DiGeorge anomaly or conditions associated with DiGeorge anomaly are prevented, detected, diagnosed, prognosticated, treated and/or ameliorated using polypeptides or polynucleotides of the invention, or antagonists or agonists thereof.

Other immunodeficiencies that may be prevented, detected, diagnosed, prognosticated, treated and/or ameliorated using polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof, include, but are not limited to, chronic granulomatous disease, Chédiak-Higashi syndrome, myeloperoxidase deficiency, leukocyte glucose-6-phosphate dehydrogenase deficiency, X-linked lymphoproliferative syndrome (XLP), leukocyte adhesion deficiency, complement component deficiencies (including C1, C2, C3, C4, C5, C6, C7, C8 and/or C9 deficiencies), reticular dysgenesis, thymic aplasia, immunodeficiency with thymoma, severe congenital leukopenia, dysplasia with immunodeficiency, neonatal neutropenia, short limbed dwarfism, and Nezelof syndrome-combined immunodeficiency with Igs.

In a preferred embodiment, the immunodeficiencies and/or conditions associated with the immunodeficiencies recited above are prevented, detected, diagnosed, prognosticated, treated and/or ameliorated using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In a preferred embodiment polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among immunodeficient individuals. In specific embodiments, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among B cell and/or T cell immunodeficient individuals.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in preventing, detecting, diagnosing, prognosticating, treating and/or ameliorating autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of polynucleotides and polypeptides of the invention that can inhibit an immune response,

particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Autoimmune diseases or disorders that may be prevented, detected, diagnosed, prognosticated, treated, and/or ameliorated by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, one or more of the following: systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis, autoimmune thyroiditis, Hashimoto's thyroiditis, autoimmune hemolytic anemia, hemolytic anemia, thrombocytopenia, autoimmune thrombocytopenia purpura, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, purpura (e.g., Henloch-Schoenlein purpura), autoimmune cytopenia, Goodpasture's syndrome, Pemphigus vulgaris, myasthenia gravis, Grave's disease (hyperthyroidism), and insulin-resistant diabetes mellitus.

Additional disorders that are likely to have an autoimmune component that may be prevented, detected, diagnosed, prognosticated, treated and/or ameliorated with the compositions of the invention include, but are not limited to, type II collagen-induced arthritis, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, neuritis, uveitis, ophthalmia, polyendocrinopathies, Reiter's Disease, Stiff-Man Syndrome, autoimmune pulmonary inflammation, autism, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disorders.

Additional disorders that are likely to have an autoimmune component that may be prevented, detected, diagnosed, prognosticated, treated and/or ameliorated with the compositions of the invention include, but are not limited to, scleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed connective tissue disease (often characterized, e.g., by antibodies to extractable nuclear antigens (e.g., ribonucleoprotein)), polymyositis (often characterized, e.g., by nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often characterized, e.g., by glomerular basement membrane antibodies or immune complexes), bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes mellitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

Additional disorders that may have an autoimmune component that may be prevented, detected, diagnosed, prognosticated, treated and/or ameliorated with the compositions of the invention include, but are not limited to, chronic active hepatitis (often characterized, e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitochondria

antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiomyopathy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), and many other inflammatory, granulomatous, degenerative, and atrophic disorders.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are prevented, detected, diagnosed, prognosticated, treated and/or ameliorated using for example, antagonists or agonists, polypeptides or polynucleotides, or antibodies of the present invention. In a specific preferred embodiment, rheumatoid arthritis is prevented, detected, diagnosed, prognosticated, treated and/or ameliorated using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In another specific preferred embodiment, systemic lupus erythematosus is prevented, detected, diagnosed, prognosticated, treated and/or ameliorated using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment, idiopathic thrombocytopenia purpura is prevented, detected, diagnosed, prognosticated, treated and/or ameliorated using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In another specific preferred embodiment IgA nephropathy is prevented, detected, diagnosed, prognosticated, treated and/or ameliorated using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are prevented, detected, diagnosed, prognosticated, treated and/or ameliorated using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention

In preferred embodiments, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a immunosuppressive agent(s).

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in detecting, preventing, diagnosing, prognosticating, treating, and/or ameliorating diseases, disorders, and/or conditions of hematopoietic cells. Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types hematopoietic cells, including but not limited to, leukopenia,

neutropenia, anemia, and thrombocytopenia. Alternatively, Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with an increase in certain (or many) types of hematopoietic cells, including but not limited to, histiocytosis.

Allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be detected, prevented, diagnosed, prognosticated, treated, and/or ameliorated using polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof. Moreover, these molecules can be used to treat, prevent, prognose, and/or diagnose anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

Additionally, polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof, may be used to detect, prevent, diagnose, prognosticate, treat, and/or ameliorate IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema. In specific embodiments, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate IgE concentrations in vitro or in vivo.

Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention have uses in the detection, prevention, diagnosis, prognostication, treatment, and/or amelioration of inflammatory conditions. For example, since polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists of the invention may inhibit the activation, proliferation and/or differentiation of cells involved in an inflammatory response, these molecules can be used to prevent and/or treat chronic and acute inflammatory conditions. Such inflammatory conditions include, but are not limited to, for example, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome), ischemia-reperfusion injury, endotoxin lethality, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, over production of cytokines (e.g., TNF or IL-1), respiratory disorders (e.g., asthma and allergy); gastrointestinal disorders (e.g., inflammatory bowel disease); cancers (e.g., gastric, ovarian, lung, bladder, liver, and breast); CNS disorders (e.g., multiple sclerosis; ischemic brain injury and/or stroke, traumatic brain injury, neurodegenerative disorders (e.g., Parkinson's disease and Alzheimer's disease); AIDS-related dementia; and prion disease); cardiovascular disorders (e.g., atherosclerosis, myocarditis, cardiovascular disease, and cardiopulmonary bypass complications); as well as many additional diseases, conditions, and disorders that are characterized by inflammation (e.g., hepatitis, rheumatoid arthritis, gout, trauma, pancreatitis, sarcoidosis, dermatitis, renal ischemia-reperfusion injury, Grave's disease, systemic lupus erythematosus, diabetes mellitus, and allogeneic transplant rejection).

Because inflammation is a fundamental defense mechanism, inflammatory disorders can

affect virtually any tissue of the body. Accordingly, polynucleotides, polypeptides, and antibodies of the invention, as well as agonists or antagonists thereof, have uses in the treatment of tissue-specific inflammatory disorders, including, but not limited to, adrenalitis, alveolitis, angiocholecystitis, appendicitis, balanitis, blepharitis, bronchitis, bursitis, carditis, cellulitis, cervicitis, cholecystitis, chondritis, cochitis, colitis, conjunctivitis, cystitis, dermatitis, diverticulitis, encephalitis, endocarditis, esophagitis, eustachitis, fibrositis, folliculitis, gastritis, gastroenteritis, gingivitis, glossitis, hepatosplenitis, keratitis, labyrinthitis, laryngitis, lymphangitis, mastitis, media otitis, meningitis, metritis, mucitis, myocarditis, myositis, myringitis, nephritis, neuritis, orchitis, osteochondritis, otitis, pericarditis, peritendonitis, peritonitis, pharyngitis, phlebitis, poliomyelitis, prostatitis, pulpitis, retinitis, rhinitis, salpingitis, scleritis, sclerochoroiditis, scrotitis, sinusitis, spondylitis, steatitis, stomatitis, synovitis, syringitis, tendonitis, tonsillitis, urethritis, and vaginitis.

In specific embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to detect, prevent, diagnose, prognosticate, treat, and/or ameliorate organ transplant rejections and graft-versus-host disease. Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. Polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD. In specific embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing experimental allergic and hyperacute xenograft rejection.

In other embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to detect, prevent, diagnose, prognosticate, treat, and/or ameliorate immune complex diseases, including, but not limited to, serum sickness, post streptococcal glomerulonephritis, polyarteritis nodosa, and immune complex-induced vasculitis.

Polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the invention can be used to treat, detect, and/or prevent infectious agents. For example, by increasing the immune response, particularly increasing the proliferation activation and/or differentiation of B and/or T cells, infectious diseases may be treated, detected, and/or prevented. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may also directly inhibit the infectious agent (refer to section of application listing infectious agents, etc), without necessarily eliciting an immune response.

In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a vaccine adjuvant that enhances immune responsiveness to an antigen. In a specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance tumor-specific immune responses.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, respiratory syncytial virus, Dengue, rotavirus, Japanese B encephalitis, influenza A and B, parainfluenza, measles, cytomegalovirus, rabies, Junin, Chikungunya, Rift Valley Fever, herpes simplex, and yellow fever.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and meningitis type B.

In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: *Vibrio cholerae*, *Mycobacterium leprae*, *Salmonella typhi*, *Salmonella paratyphi*, *Meissneria meningitidis*, *Streptococcus pneumoniae*, Group B streptococcus, *Shigella spp.*, Enterotoxigenic *Escherichia coli*, Enterohemorrhagic *E. coli*, and *Borrelia burgdorferi*.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune

response to Plasmodium (malaria) or Leishmania.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed to treat infectious diseases including silicosis, sarcoidosis, and idiopathic pulmonary fibrosis; for example, by preventing the recruitment and activation of mononuclear phagocytes.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an antigen for the generation of antibodies to inhibit or enhance immune mediated responses against polypeptides of the invention.

In one embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are administered to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production and immunoglobulin class switching (e.g., IgG, IgA, IgM, and IgE), and/or to increase an immune response.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a stimulator of B cell responsiveness to pathogens.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an activator of T cells.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent that elevates the immune status of an individual prior to their receipt of immunosuppressive therapies.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to induce higher affinity antibodies.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to increase serum immunoglobulin concentrations.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to accelerate recovery of immunocompromised individuals.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among aged populations and/or neonates.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ

transplantation). With respect to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific embodiment, compositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but prior to full recovery of B cell populations.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, HIV Infection, AIDS, bone marrow transplant, and B cell chronic lymphocytic leukemia (CLL).

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, and recovery from surgery.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention enhance antigen presentation or antagonizes antigen presentation in vitro or in vivo. Moreover, in related embodiments, said enhancement or antagonism of antigen presentation may be useful as an anti-tumor treatment or to modulate the immune system.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to direct an individual's immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists

or antagonists of the present invention are used as a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodeficiency.

5 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect. In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used in the pretreatment of bone marrow samples prior to transplant.

10 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a gene-based therapy for genetically inherited disorders resulting in immuno-incompetence/immunodeficiency such as observed among SCID patients.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leishmania.

15 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of regulating secreted cytokines that are elicited by polypeptides of the invention.

20 In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used in one or more of the applications described herein, as they may apply to veterinary medicine.

25 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of blocking various aspects of immune responses to foreign agents or self. Examples of diseases or conditions in which blocking of certain aspects of immune responses may be desired include autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and diseases/disorders associated with pathogens.

30 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for preventing the B cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythematosus and multiple sclerosis.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a inhibitor of B and/or T cell migration in endothelial cells. This activity disrupts tissue architecture or cognate responses and is useful, for example in disrupting immune responses, and blocking sepsis.

35 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for chronic hypergammaglobulinemia evident in such diseases as monoclonal gammopathy of undetermined significance (MGUS),

Waldenstrom's disease, related idiopathic monoclonal gammopathies, and plasmacytomas.

5 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed for instance to inhibit polypeptide chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain autoimmune and chronic inflammatory and infective diseases. Examples of autoimmune diseases are described herein and include multiple sclerosis, and insulin-dependent diabetes.

10 The polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed to treat idiopathic hyper-eosinophilic syndrome by, for example, preventing eosinophil production and migration.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used to enhance or inhibit complement mediated cell lysis.

15 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used to enhance or inhibit antibody dependent cellular cytotoxicity.

20 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed for treating atherosclerosis, for example, by preventing monocyte infiltration in the artery wall.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed to treat adult respiratory distress syndrome (ARDS).

25 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be useful for stimulating wound and tissue repair, stimulating angiogenesis, and/or stimulating the repair of vascular or lymphatic diseases or disorders. Additionally, agonists and antagonists of the invention may be used to stimulate the regeneration of mucosal surfaces.

30 In a specific embodiment, polynucleotides or polypeptides, and/or agonists thereof are used to detect, prevent, diagnose, prognosticate, treat, and/or ameliorate a disorder characterized by primary or acquired immunodeficiency, deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, polynucleotides or polypeptides, and/or agonists thereof may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis),
35 autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV

disease, CLL, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carinii. Other diseases and disorders that may be detected, prevented, diagnosed, prognosticated, treated, and/or ameliorated with polynucleotides or polypeptides, and/or agonists of the present invention include, but are not limited to, HIV infection, HTLV-BLV infection, lymphopenia, phagocyte bactericidal dysfunction anemia, thrombocytopenia, and hemoglobinuria.

In another embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention are used to treat, and/or diagnose an individual having common variable immunodeficiency disease ("CVID"; also known as "acquired agammaglobulinemia" and "acquired hypogammaglobulinemia") or a subset of this disease.

In a specific embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to detect, prevent, diagnose, prognosticate, treat, and/or ameliorate cancers or neoplasms including immune cell or immune tissue-related cancers or neoplasms. Examples of cancers or neoplasms that may be detected, prevented, diagnosed, prognosticated, treated, and/or ameliorated by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, acute myelogenous leukemia, chronic myelogenous leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, acute lymphocytic anemia (ALL) Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, EBV-transformed diseases, and/or diseases and disorders described in the section entitled "Hyperproliferative Disorders" elsewhere herein.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for decreasing cellular proliferation of Large B-cell Lymphomas.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

In specific embodiments, the compositions of the invention are used as an agent to boost immunoresponsiveness among B cell immunodeficient individuals, such as, for example, an individual who has undergone a partial or complete splenectomy.

Antagonists of the invention include, for example, binding and/or inhibitory antibodies, antisense nucleic acids, ribozymes or soluble forms of the polypeptides of the present invention (e.g., Fc fusion protein; see, e.g., Example 9). Agonists of the invention include, for example, binding or stimulatory antibodies, and soluble forms of the polypeptides (e.g., Fc fusion proteins; see, e.g., Example 9), polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or

antagonists of the present invention are administered to an animal (including, but not limited to, those listed above, and also including transgenic animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT Application Nos. WO98/24893, WO/9634096, WO/9633735, and WO/9110741). Administration of polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention to such animals is useful for the generation of monoclonal antibodies against the polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention.

Respiratory Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may be used to detect, prevent, diagnose, prognosticate, treat, and/or ameliorate diseases and/or disorders of the respiratory system.

Diseases and disorders of the respiratory system include, but are not limited to, nasal vestibulitis, nonallergic rhinitis (e.g., acute rhinitis, chronic rhinitis, atrophic rhinitis, vasomotor rhinitis), nasal polyps, and sinusitis, juvenile angiofibromas, cancer of the nose and juvenile papillomas, vocal cord polyps, nodules (singer's nodules), contact ulcers, vocal cord paralysis, laryngoceles, pharyngitis (e.g., viral and bacterial), tonsillitis, tonsillar cellulitis, parapharyngeal abscess, laryngitis, laryngoceles, and throat cancers (e.g., cancer of the nasopharynx, tonsil cancer, larynx cancer), lung cancer (e.g., squamous cell carcinoma, small cell (oat cell) carcinoma, large cell carcinoma, and adenocarcinoma), allergic disorders (eosinophilic pneumonia, hypersensitivity pneumonitis (e.g., extrinsic allergic alveolitis, allergic interstitial pneumonitis, organic dust pneumoconiosis, allergic bronchopulmonary aspergillosis, asthma, Wegener's granulomatosis (granulomatous vasculitis), Goodpasture's syndrome)), pneumonia (e.g., bacterial pneumonia (e.g., *Streptococcus pneumoniae* (pneumococcal pneumonia), *Staphylococcus aureus* (staphylococcal pneumonia), Gram-negative bacterial pneumonia (caused by, e.g., *Klebsiella* and *Pseudomonas spp.*), *Mycoplasma pneumoniae* pneumonia, *Hemophilus influenzae* pneumonia, *Legionella pneumophila* (Legionnaires' disease), and *Chlamydia psittaci* (Psittacosis)), and viral pneumonia (e.g., influenza, chickenpox (varicella).

Additional diseases and disorders of the respiratory system include, but are not limited to bronchiolitis, polio (poliomyelitis), croup, respiratory syncytial viral infection, mumps, erythema infectiosum (fifth disease), roseola infantum, progressive rubella panencephalitis, german measles, and subacute sclerosing panencephalitis), fungal pneumonia (e.g., Histoplasmosis, Coccidioidomycosis, Blastomycosis, fungal infections in people with severely suppressed immune systems (e.g., cryptococcosis, caused by *Cryptococcus neoformans*; aspergillosis, caused by

Aspergillus spp.; candidiasis, caused by *Candida*; and mucormycosis)), *Pneumocystis carinii* (pneumocystis pneumonia), atypical pneumonias (e.g., *Mycoplasma* and *Chlamydia* spp.), opportunistic infection pneumonia, nosocomial pneumonia, chemical pneumonitis, and aspiration pneumonia, pleural disorders (e.g., pleurisy, pleural effusion, and pneumothorax (e.g., simple spontaneous pneumothorax, complicated spontaneous pneumothorax, tension pneumothorax)), obstructive airway diseases (e.g., asthma, chronic obstructive pulmonary disease (COPD), emphysema, chronic or acute bronchitis), occupational lung diseases (e.g., silicosis, black lung (coal workers' pneumoconiosis), asbestosis, berylliosis, occupational asthma, byssinosis, and benign pneumoconioses), Infiltrative Lung Disease (e.g., pulmonary fibrosis (e.g., fibrosing alveolitis, usual interstitial pneumonia), idiopathic pulmonary fibrosis, desquamative interstitial pneumonia, lymphoid interstitial pneumonia, histiocytosis X (e.g., Letterer-Siwe disease, Hand-Schüller-Christian disease, eosinophilic granuloma), idiopathic pulmonary hemosiderosis, sarcoidosis and pulmonary alveolar proteinosis), Acute respiratory distress syndrome (also called, e.g., adult respiratory distress syndrome), edema, pulmonary embolism, bronchitis (e.g., viral, bacterial), bronchiectasis, atelectasis, lung abscess (caused by, e.g., *Staphylococcus aureus* or *Legionella pneumophila*), and cystic fibrosis.

Wound Healing and Epithelial Cell Proliferation

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote dermal reestablishment subsequent to dermal loss

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are types of grafts that polynucleotides or polypeptides, agonists or antagonists of the present invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepdermic grafts,

avascular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omentoplastic graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft.

5 Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine.

10 Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. Polynucleotides or polypeptides, agonists or antagonists of the present invention, may promote proliferation of endothelial cells, 15 keratinocytes, and basal keratinocytes.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may have a cytoprotective effect on the small intestine 20 mucosa. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could further be used in full regeneration of skin in full and partial thickness skin 25 defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. Polynucleotides or 30 polypeptides, as well as agonists or antagonists of the present invention, could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, polynucleotides or 35 polypeptides, as well as agonists or antagonists of the present invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with polynucleotides or polypeptides, agonists or

antagonists of the present invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat diseases associate
5 with the under expression.

Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to prevent and heal damage to the lungs due to various pathological states. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, which could stimulate proliferation and differentiation and promote the repair of
10 alveoli and brochiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of aveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated using polynucleotides or polypeptides, agonists or antagonists of the present invention. Also, polynucleotides or polypeptides, as well as agonists or antagonists of the
15 present invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary displasia, in premature infants.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be
20 used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetraholoride and other hepatotoxins known in the art).

In addition, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used treat or prevent the onset of diabetes mellitus. In patients with
25 newly diagnosed Types I and II diabetes, where some islet cell function remains, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell
30 function.

Gastrointestinal Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, may be used to detect, prevent, diagnose, prognosticate, treat, and/or ameliorate gastrointestinal
35 diseases and disorders, including inflammatory diseases and/or conditions, infections, cancers (e.g., intestinal neoplasms (carcinoid tumor of the small intestine, non-Hodgkin's lymphoma of the small intestine, small bowl lymphoma)), and ulcers, such as peptic ulcers.

Gastrointestinal disorders include dysphagia, odynophagia, inflammation of the esophagus, peptic esophagitis, gastric reflux, submucosal fibrosis and stricturing, Mallory-Weiss lesions, leiomyomas, lipomas, epidermal cancers, adeoncarcinomas, gastric retention disorders, gastroenteritis, gastric atrophy, gastric/stomach cancers, polyps of the stomach, autoimmune disorders such as pernicious anemia, pyloric stenosis, gastritis (bacterial, viral, eosinophilic, stress-induced, chronic erosive, atrophic, plasma cell, and Ménétrier's), and peritoneal diseases (e.g., chyloperitoneum, hemoperitoneum, mesenteric cyst, mesenteric lymphadenitis, mesenteric vascular occlusion, panniculitis, neoplasms, peritonitis, pneumoperitoneum, bubphrenic abscess.).

Gastrointestinal disorders also include disorders associated with the small intestine, such as malabsorption syndromes, distension, irritable bowel syndrome, sugar intolerance, celiac disease, duodenal ulcers, duodenitis, tropical sprue, Whipple's disease, intestinal lymphangiectasia, Crohn's disease, appendicitis, obstructions of the ileum, Meckel's diverticulum, multiple diverticula, failure of complete rotation of the small and large intestine, lymphoma, and bacterial and parasitic diseases (such as Traveler's diarrhea, typhoid and paratyphoid, cholera, infection by Roundworms (*Ascariasis lumbricoides*), Hookworms (*Ancylostoma duodenale*), Threadworms (*Enterobius vermicularis*), Tapeworms (*Taenia saginata*, *Echinococcus granulosus*, *Diphyllobothrium spp.*, and *T. solium*).

Liver diseases and/or disorders include intrahepatic cholestasis (alagille syndrome, biliary liver cirrhosis), fatty liver (alcoholic fatty liver, reye syndrome), hepatic vein thrombosis, hepatolenticular degeneration, hepatomegaly, hepatopulmonary syndrome, hepatorenal syndrome, portal hypertension (esophageal and gastric varices), liver abscess (amebic liver abscess), liver cirrhosis (alcoholic, biliary and experimental), alcoholic liver diseases (fatty liver, hepatitis, cirrhosis), parasitic (hepatic echinococcosis, fascioliasis, amebic liver abscess), jaundice (hemolytic, hepatocellular, and cholestatic), cholestasis, portal hypertension, liver enlargement, ascites, hepatitis (alcoholic hepatitis, animal hepatitis, chronic hepatitis (autoimmune, hepatitis B, hepatitis C, hepatitis D, drug induced), toxic hepatitis, viral human hepatitis (hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E), Wilson's disease, granulomatous hepatitis, secondary biliary cirrhosis, hepatic encephalopathy, portal hypertension, varices, hepatic encephalopathy, primary biliary cirrhosis, primary sclerosing cholangitis, hepatocellular adenoma, hemangiomas, bile stones, liver failure (hepatic encephalopathy, acute liver failure), and liver neoplasms (angiomyolipoma, calcified liver metastases, cystic liver metastases, epithelial tumors, fibrolamellar hepatocarcinoma, focal nodular hyperplasia, hepatic adenoma, hepatobiliary cystadenoma, hepatoblastoma, hepatocellular carcinoma, hepatoma, liver cancer, liver hemangioendothelioma, mesenchymal hamartoma, mesenchymal tumors of liver, nodular regenerative hyperplasia, benign liver tumors (Hepatic cysts [Simple cysts, Polycystic liver disease, Hepatobiliary cystadenoma, Choledochal cyst], Mesenchymal tumors [Mesenchymal hamartoma, Infantile hemangioendothelioma, Hemangioma, Peliosis hepatis, Lipomas,

Inflammatory pseudotumor, Miscellaneous], Epithelial tumors [Bile duct epithelium (Bile duct hamartoma, Bile duct adenoma), Hepatocyte (Adenoma, Focal nodular hyperplasia, Nodular regenerative hyperplasia)], malignant liver tumors [hepatocellular, hepatoblastoma, hepatocellular carcinoma, cholangiocellular, cholangiocarcinoma, cystadenocarcinoma, tumors of blood vessels, angiosarcoma, Kaposi's sarcoma, hemangioendothelioma, other tumors, embryonal sarcoma, fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, carcinosarcoma, teratoma, carcinoid, squamous carcinoma, primary lymphoma]), peliosis hepatis, erythrohepatic porphyria, hepatic porphyria (acute intermittent porphyria, porphyria cutanea tarda), Zellweger syndrome).

Pancreatic diseases and/or disorders include acute pancreatitis, chronic pancreatitis (acute necrotizing pancreatitis, alcoholic pancreatitis), neoplasms (adenocarcinoma of the pancreas, cystadenocarcinoma, insulinoma, gastrinoma, and glucagonoma, cystic neoplasms, islet-cell tumors, pancreoblastoma), and other pancreatic diseases (e.g., cystic fibrosis, cyst (pancreatic pseudocyst, pancreatic fistula, insufficiency)).

Gallbladder diseases include gallstones (cholelithiasis and choledocholithiasis), postcholecystectomy syndrome, diverticulosis of the gallbladder, acute cholecystitis, chronic cholecystitis, bile duct tumors, and mucocele.

Diseases and/or disorders of the large intestine include antibiotic-associated colitis, diverticulitis, ulcerative colitis, acquired megacolon, abscesses, fungal and bacterial infections, anorectal disorders (e.g., fissures, hemorrhoids), colonic diseases (colitis, colonic neoplasms [colon cancer, adenomatous colon polyps (e.g., villous adenoma), colon carcinoma, colorectal cancer], colonic diverticulitis, colonic diverticulosis, megacolon [Hirschsprung disease, toxic megacolon]; sigmoid diseases [proctocolitis, sigmoid neoplasms]), constipation, Crohn's disease, diarrhea (infantile diarrhea, dysentery), duodenal diseases (duodenal neoplasms, duodenal obstruction, duodenal ulcer, duodenitis), enteritis (enterocolitis), HIV enteropathy, ileal diseases (ileal neoplasms, ileitis), immunoproliferative small intestinal disease, inflammatory bowel disease (ulcerative colitis, Crohn's disease), intestinal atresia, parasitic diseases (anisakiasis, balantidiasis, blastocystis infections, cryptosporidiosis, dientamoebiasis, amebic dysentery, giardiasis), intestinal fistula (rectal fistula), intestinal neoplasms (cecal neoplasms, colonic neoplasms, duodenal neoplasms, ileal neoplasms, intestinal polyps, jejunal neoplasms, rectal neoplasms), intestinal obstruction (afferent loop syndrome, duodenal obstruction, impacted feces, intestinal pseudo-obstruction [cecal volvulus], intussusception), intestinal perforation, intestinal polyps (colonic polyps, gardner syndrome, peutz-jeghers syndrome), jejunal diseases (jejunal neoplasms), malabsorption syndromes (blind loop syndrome, celiac disease, lactose intolerance, short bowel syndrome, tropical sprue, whipple's disease), mesenteric vascular occlusion, pneumatosis cystoides intestinalis, protein-losing enteropathies (intestinal lymphagiectasis), rectal diseases (anus diseases, fecal incontinence, hemorrhoids, proctitis, rectal fistula, rectal prolapse, rectocele), peptic ulcer (duodenal ulcer, peptic esophagitis, hemorrhage, perforation, stomach ulcer,

Zollinger-Ellison syndrome), postgastrectomy syndromes (dumping syndrome), stomach diseases (e.g., achlorhydria, duodenogastric reflux (bile reflux), gastric antral vascular ectasia, gastric fistula, gastric outlet obstruction, gastritis (atrophic or hypertrophic), gastroparesis, stomach dilatation, stomach diverticulum, stomach neoplasms (gastric cancer, gastric polyps, gastric adenocarcinoma, hyperplastic gastric polyp), stomach rupture, stomach ulcer, stomach volvulus), tuberculosis, visceroptosis, vomiting (e.g., hematemesis, hyperemesis gravidarum, postoperative nausea and vomiting) and hemorrhagic colitis.

Further diseases and/or disorders of the gastrointestinal system include biliary tract diseases, such as, gastroschisis, fistula (e.g., biliary fistula, esophageal fistula, gastric fistula, intestinal fistula, pancreatic fistula), neoplasms (e.g., biliary tract neoplasms, esophageal neoplasms, such as adenocarcinoma of the esophagus, esophageal squamous cell carcinoma, gastrointestinal neoplasms, pancreatic neoplasms, such as adenocarcinoma of the pancreas, mucinous cystic neoplasm of the pancreas, pancreatic cystic neoplasms, pancreatoblastoma, and peritoneal neoplasms), esophageal disease (e.g., bullous diseases, candidiasis, glycogenic acanthosis, ulceration, barrett esophagus varices, atresia, cyst, diverticulum (e.g., Zenker's diverticulum), fistula (e.g., tracheoesophageal fistula), motility disorders (e.g., CREST syndrome, deglutition disorders, achalasia, spasm, gastroesophageal reflux), neoplasms, perforation (e.g., Boerhaave syndrome, Mallory-Weiss syndrome), stenosis, esophagitis, diaphragmatic hernia (e.g., hiatal hernia); gastrointestinal diseases, such as, gastroenteritis (e.g., cholera morbus, norwalk virus infection), hemorrhage (e.g., hematemesis, melena, peptic ulcer hemorrhage), stomach neoplasms (gastric cancer, gastric polyps, gastric adenocarcinoma, stomach cancer)), hernia (e.g., congenital diaphragmatic hernia, femoral hernia, inguinal hernia, obturator hernia, umbilical hernia, ventral hernia), and intestinal diseases (e.g., cecal diseases (appendicitis, cecal neoplasms)).

Chemotaxis

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting

immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could be used as an inhibitor of chemotaxis.

Binding Activity

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991)). Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which the polypeptide of the present invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labeled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to auto-radiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of the polypeptide of the present invention thereby effectively generating agonists and antagonists of the polypeptide of the present invention. *See generally*, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., et al., *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., et al., *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *Biotechniques* 24(2):308-13 (1998); each of these patents and publications are hereby incorporated by reference).

In one embodiment, alteration of polynucleotides and corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptide of the present invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous

molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptide of the present invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and $^3\text{[H]}$ thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of $^3\text{[H]}$ thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of $^3\text{[H]}$ thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the

polypeptides of the invention from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a polypeptide of the present invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a polypeptide of the present invention, (b) assaying a biological activity, and (b) determining if a biological activity of the polypeptide has been altered.

Targeted Delivery

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound.

Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

5

Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells that are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other agents that affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition,

non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

Antisense And Ribozyme (Antagonists)

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to cDNA sequences contained in cDNA ATCC Deposit No:Z identified for example, in Table 1A and/or 1B. In one embodiment, antisense sequence is generated internally, by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, J., Neurochem. 56:560 (1991). Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, J., Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., Nucleic Acids Research 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed *in vitro* by incubating cells with the oligoribonucleotide. A similar procedure for *in vivo* use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoR1 site on the 5' end and a HindIII site on the 3' end. Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl₂, 10mM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoR1/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the

mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding the polypeptide of the present invention or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature* 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, et al., *Nature* 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of the present invention. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, *Nature* 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non- translated, non-coding regions of polynucleotide sequences described herein could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the

invention. Whether designed to hybridize to the 5'-, 3'- or coding region of mRNA of the present invention, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents. (See, e.g., Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

While antisense nucleotides complementary to the coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of SEQ ID NO:X. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.) and should be delivered to cells which express *in vivo*. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth and

proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

5 The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirable in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

10 The antagonist/agonist may also be employed to treat the diseases described herein.

Thus, the invention provides a method of treating disorders or diseases, including but not limited to the disorders or diseases listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme
15 directed to the polynucleotide of the present invention.

Binding Peptides and Other Molecules

The invention also encompasses screening methods for identifying polypeptides and nonpolypeptides that bind polypeptides of the invention, and the binding molecules identified
20 thereby. These binding molecules are useful, for example, as agonists and antagonists of the polypeptides of the invention. Such agonists and antagonists can be used, in accordance with the invention, in the therapeutic embodiments described in detail, below.

This method comprises the steps of:

contacting polypeptides of the invention with a plurality of molecules; and
25 identifying a molecule that binds the polypeptides of the invention.

The step of contacting the polypeptides of the invention with the plurality of molecules may be effected in a number of ways. For example, one may contemplate immobilizing the polypeptides on a solid support and bringing a solution of the plurality of molecules in contact with the immobilized polypeptides. Such a procedure would be akin to an affinity
30 chromatographic process, with the affinity matrix being comprised of the immobilized polypeptides of the invention. The molecules having a selective affinity for the polypeptides can then be purified by affinity selection. The nature of the solid support, process for attachment of the polypeptides to the solid support, solvent, and conditions of the affinity isolation or selection are largely conventional and well known to those of ordinary skill in the art.

35 Alternatively, one may also separate a plurality of polypeptides into substantially separate fractions comprising a subset of or individual polypeptides. For instance, one can separate the plurality of polypeptides by gel electrophoresis, column chromatography, or like method known to

those of ordinary skill for the separation of polypeptides. The individual polypeptides can also be produced by a transformed host cell in such a way as to be expressed on or about its outer surface (e.g., a recombinant phage). Individual isolates can then be "probed" by the polypeptides of the invention, optionally in the presence of an inducer should one be required for expression, to determine if any selective affinity interaction takes place between the polypeptides and the individual clone. Prior to contacting the polypeptides with each fraction comprising individual polypeptides, the polypeptides could first be transferred to a solid support for additional convenience. Such a solid support may simply be a piece of filter membrane, such as one made of nitrocellulose or nylon. In this manner, positive clones could be identified from a collection of transformed host cells of an expression library, which harbor a DNA construct encoding a polypeptide having a selective affinity for polypeptides of the invention. Furthermore, the amino acid sequence of the polypeptide having a selective affinity for the polypeptides of the invention can be determined directly by conventional means or the coding sequence of the DNA encoding the polypeptide can frequently be determined more conveniently. The primary sequence can then be deduced from the corresponding DNA sequence. If the amino acid sequence is to be determined from the polypeptide itself, one may use microsequencing techniques. The sequencing technique may include mass spectroscopy.

In certain situations, it may be desirable to wash away any unbound polypeptides from a mixture of the polypeptides of the invention and the plurality of polypeptides prior to attempting to determine or to detect the presence of a selective affinity interaction. Such a wash step may be particularly desirable when the polypeptides of the invention or the plurality of polypeptides are bound to a solid support.

The plurality of molecules provided according to this method may be provided by way of diversity libraries, such as random or combinatorial peptide or nonpeptide libraries which can be screened for molecules that specifically bind polypeptides of the invention. Many libraries are known in the art that can be used, e.g., chemically synthesized libraries, recombinant (e.g., phage display libraries), and in vitro translation-based libraries. Examples of chemically synthesized libraries are described in Fodor et al., 1991, *Science* 251:767-773; Houghten et al., 1991, *Nature* 354:84-86; Lam et al., 1991, *Nature* 354:82-84; Medynski, 1994, *Bio/Technology* 12:709-710; Gallop et al., 1994, *J. Medicinal Chemistry* 37(9):1233-1251; Ohlmeyer et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:10922-10926; Erb et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:11422-11426; Houghten et al., 1992, *Biotechniques* 13:412; Jayawickreme et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:1614-1618; Salmon et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:11708-11712; PCT Publication No. WO 93/20242; and Brenner and Lerner, 1992, *Proc. Natl. Acad. Sci. USA* 89:5381-5383.

Examples of phage display libraries are described in Scott and Smith, 1990, *Science* 249:386-390; Devlin et al., 1990, *Science*, 249:404-406; Christian, R. B., et al., 1992, *J. Mol. Biol.*

227:711-718); Lenstra, 1992, *J. Immunol. Meth.* 152:149-157; Kay et al., 1993, *Gene* 128:59-65; and PCT Publication No. WO 94/18318 dated Aug. 18, 1994.

In vitro translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/05058 dated Apr. 18, 1991; and Mattheakis et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:9022-9026.

By way of examples of nonpeptide libraries, a benzodiazepine library (see e.g., Bunin et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:4708-4712) can be adapted for use. Peptoid libraries (Simon et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:9367-9371) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al. (1994, *Proc. Natl. Acad. Sci. USA* 91:11138-11142).

The variety of non-peptide libraries that are useful in the present invention is great. For example, Ecker and Crooke, 1995, *Bio/Technology* 13:351-360 list benzodiazepines, hydantoins, piperazinediones, biphenyls, sugar analogs, beta-mercaptoketones, arylacetic acids, acylpiperidines, benzopyrans, cubanes, xanthines, aminimides, and oxazolones as among the chemical species that form the basis of various libraries.

Non-peptide libraries can be classified broadly into two types: decorated monomers and oligomers. Decorated monomer libraries employ a relatively simple scaffold structure upon which a variety functional groups is added. Often the scaffold will be a molecule with a known useful pharmacological activity. For example, the scaffold might be the benzodiazepine structure.

Non-peptide oligomer libraries utilize a large number of monomers that are assembled together in ways that create new shapes that depend on the order of the monomers. Among the monomer units that have been used are carbamates, pyrrolinones, and morpholinos. Peptoids, peptide-like oligomers in which the side chain is attached to the alpha amino group rather than the alpha carbon, form the basis of another version of non-peptide oligomer libraries. The first non-peptide oligomer libraries utilized a single type of monomer and thus contained a repeating backbone. Recent libraries have utilized more than one monomer, giving the libraries added flexibility.

Screening the libraries can be accomplished by any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of peptide libraries: Parmley and Smith, 1989, *Adv. Exp. Med. Biol.* 251:215-218; Scott and Smith, 1990, *Science* 249:386-390; Fowlkes et al., 1992, *BioTechniques* 13:422-427; Oldenburg et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:5393-5397; Yu et al., 1994, *Cell* 76:933-945; Staudt et al., 1988, *Science* 241:577-580; Bock et al., 1992, *Nature* 355:564-566; Tuerk et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:6988-6992; Ellington et al., 1992, *Nature* 355:850-852; U.S. Pat. No. 5,096,815, U.S. Pat. No. 5,223,409, and U.S. Pat. No. 5,198,346, all to Ladner et al.; Rebar and Pabo, 1993, *Science* 263:671-673; and CT Publication No. WO 94/18318.

In a specific embodiment, screening to identify a molecule that binds polypeptides of the invention can be carried out by contacting the library members with polypeptides of the invention immobilized on a solid phase and harvesting those library members that bind to the polypeptides of the invention. Examples of such screening methods, termed "panning" techniques are described by way of example in Parmley and Smith, 1988, Gene 73:305-318; Fowlkes et al., 1992, BioTechniques 13:422-427; PCT Publication No. WO 94/18318; and in references cited herein.

In another embodiment, the two-hybrid system for selecting interacting proteins in yeast (Fields and Song, 1989, Nature 340:245-246; Chien et al., 1991, Proc. Natl. Acad. Sci. USA 88:9578-9582) can be used to identify molecules that specifically bind to polypeptides of the invention.

Where the binding molecule is a polypeptide, the polypeptide can be conveniently selected from any peptide library, including random peptide libraries, combinatorial peptide libraries, or biased peptide libraries. The term "biased" is used herein to mean that the method of generating the library is manipulated so as to restrict one or more parameters that govern the diversity of the resulting collection of molecules, in this case peptides.

Thus, a truly random peptide library would generate a collection of peptides in which the probability of finding a particular amino acid at a given position of the peptide is the same for all 20 amino acids. A bias can be introduced into the library, however, by specifying, for example, that a lysine occur every fifth amino acid or that positions 4, 8, and 9 of a decapeptide library be fixed to include only arginine. Clearly, many types of biases can be contemplated, and the present invention is not restricted to any particular bias. Furthermore, the present invention contemplates specific types of peptide libraries, such as phage displayed peptide libraries and those that utilize a DNA construct comprising a lambda phage vector with a DNA insert.

As mentioned above, in the case of a binding molecule that is a polypeptide, the polypeptide may have about 6 to less than about 60 amino acid residues, preferably about 6 to about 10 amino acid residues, and most preferably, about 6 to about 22 amino acids. In another embodiment, a binding polypeptide has in the range of 15-100 amino acids, or 20-50 amino acids.

The selected binding polypeptide can be obtained by chemical synthesis or recombinant expression.

Other Activities

A polypeptide, polynucleotide, agonist, or antagonist of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. The polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

5 A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed stimulate neuronal growth and to treat and prevent neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. A polypeptide, polynucleotide, agonist, or antagonist of the present invention may have the ability to stimulate chondrocyte growth,
10 therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be
15 employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, a polypeptide, polynucleotide, agonist, or antagonist of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be
20 employed to maintain organs before transplantation or for supporting cell culture of primary tissues. A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also
25 increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, a polypeptide, polynucleotide, agonist, or antagonist of the present invention may be
30 used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, cardiac rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive
35 capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be

used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific
5 embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

10 **Other Preferred Embodiments**

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, the nucleotide sequence as defined in column 5 of Table 1B or
15 columns 8 and 9 of Table 2 or the complementary strand thereto, and/or cDNA contained in ATCC Deposit No:Z.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of the portion of SEQ ID NO:X as defined in column 5, “ORF (From-To)”, in Table 1B.1.

20 Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of the portion of SEQ ID NO:X as defined in columns 8 and 9, “NT From” and “NT To” respectively, in Table 2.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the
25 nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, the nucleotide sequence as defined in column 5 of Table 1B.1 or columns 8 and 9 of Table 2 or the complementary strand thereto, and/or cDNA contained in ATCC Deposit No:Z.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the
30 nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, the nucleotide sequence as defined in column 5 of Table 1B.1 or columns 8 and 9 of Table 2 or the complementary strand thereto, and/or cDNA contained in ATCC Deposit No:Z.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of the portion of SEQ ID
35 NO:X defined in column 5, “ORF (From-To)”, in Table 1B.1.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of the portion of SEQ ID

NO:X defined in columns 8 and 9, "NT From" and "NT To", respectively, in Table 2.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, the nucleotide sequence as defined in column 5 of Table 1B.1 or columns 8 and 9 of Table 2 or the complementary strand thereto, and/or cDNA contained in ATCC Deposit No:Z.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, the nucleotide sequence as defined in column 5 of Table 1B.1 or columns 8 and 9 of Table 2 or the complementary strand thereto, and/or cDNA contained in ATCC Deposit No:Z, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises the cDNA contained in ATCC Deposit No:Z.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides of the cDNA sequence contained in ATCC Deposit No:Z.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of an open reading frame sequence encoded by cDNA contained in ATCC Deposit No:Z.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by cDNA contained in ATCC Deposit No:Z.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by cDNA contained in ATCC Deposit No:Z.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by cDNA contained in ATCC Deposit No:Z.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; the nucleotide sequence as defined in column 5 of Table 1B.1 or columns 8 and 9 of Table 2 or the complementary strand thereto; and a nucleotide sequence encoded by cDNA contained in ATCC Deposit No:Z; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule

in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; the nucleotide sequence as defined in column 5 of Table 1B.1 or columns 8 and 9 of Table 2 or the complementary strand thereto; and a nucleotide sequence of the cDNA contained in ATCC Deposit No:Z.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; the nucleotide sequence as defined in column 5 of Table 1B.1 or columns 8 and 9 of Table 2 or the complementary strand thereto; or the cDNA contained in ATCC Deposit No:Z which encodes a protein, wherein the method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; the nucleotide sequence as defined in column 5 of Table 1B.1 or columns 8 and 9 of Table 2 or the complementary strand thereto; and a nucleotide sequence of cDNA contained in ATCC Deposit No:Z.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules

wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; the nucleotide sequence as defined in column 5 of Table 1B.1 or columns 8 and 9 of Table 2 or the complementary strand thereto; and a nucleotide sequence encoded by cDNA contained in ATCC Deposit No:Z. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a DNA microarray or "chip" of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 100, 150, 200, 250, 300, 500, 1000, 2000, 3000, or 4000 nucleotide sequences, wherein at least one sequence in said DNA microarray or "chip" is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1A and/or 1B; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA "Clone ID" in Table 1A and/or 1B.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto; the polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2; and/or a polypeptide encoded by cDNA contained in ATCC Deposit No:Z.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto; the polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2; and/or a polypeptide encoded by cDNA contained in ATCC Deposit No:Z.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto; the polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2; and/or a polypeptide encoded by cDNA contained in ATCC Deposit No:Z.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto; the polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2; and/or a polypeptide encoded by cDNA contained in ATCC Deposit No:Z.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino

acid sequence of a polypeptide encoded by contained in ATCC Deposit No:Z

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a portion of said polypeptide encoded by cDNA contained in ATCC Deposit No:Z; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto; the polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2; and/or the polypeptide sequence of SEQ ID NO:Y.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of a polypeptide encoded by cDNA contained in ATCC Deposit No:Z.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto; the polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2; and a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto; the polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2; and a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ

ID NO:X or the complementary strand thereto; the polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2; and a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto; the polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2; and a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a nucleic acid sequence identified in Table 1A, 1B or Table 2 encoding a polypeptide, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto; the polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2; and a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto; the polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2; and a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is a polypeptide molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a
5 polypeptide encoded by SEQ ID NO:X or the complementary strand thereto; the polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2; and a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z.

Further preferred is a method of making a recombinant vector comprising inserting any of
10 the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this
15 recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a human protein comprising an amino acid sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto; the
20 polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2; and a polypeptide encoded by the cDNA contained in ATCC Deposit No:Z. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a protein activity, which method comprises administering to such an individual a Therapeutic
25 comprising an amount of an isolated polypeptide, polynucleotide, immunogenic fragment or analogue thereof, binding agent, antibody, or antigen binding fragment of the claimed invention effective to increase the level of said protein activity in said individual.

Also preferred is a method of treatment of an individual in need of a decreased level of a protein activity, which method comprised administering to such an individual a Therapeutic
30 comprising an amount of an isolated polypeptide, polynucleotide, immunogenic fragment or analogue thereof, binding agent, antibody, or antigen binding fragment of the claimed invention effective to decrease the level of said protein activity in said individual.

Also preferred is a method of treatment of an individual in need of a specific delivery of toxic compositions to diseased cells (e.g., tumors, leukemias or lymphomas), which method
35 comprises administering to such an individual a Therapeutic comprising an amount of an isolated polypeptide of the invention, including, but not limited to a binding agent, or antibody of the claimed invention that are associated with toxin or cytotoxic prodrugs.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

5 Description of Table 6

Table 6 summarizes some of the ATCC Deposits, Deposit dates, and ATCC designation numbers of deposits made with the ATCC in connection with the present application. These deposits were made in addition to those described in the Table 1A.

Table 6

ATCC Deposits	Deposit Date	ATCC Designation Number
LP01, LP02, LP03, LP04, LP05, LP06, LP07, LP08, LP09, LP10, LP11,	May-20-97	209059, 209060, 209061, 209062, 209063, 209064, 209065, 209066, 209067, 209068, 209069
LP12	Jan-12-98	209579
LP13	Jan-12-98	209578
LP14	Jul-16-98	203067
LP15	Jul-16-98	203068
LP16	Feb-1-99	203609
LP17	Feb-1-99	203610
LP20	Nov-17-98	203485
LP21	Jun-18-99	PTA-252
LP22	Jun-18-99	PTA-253
LP23	Dec-22-99	PTA-1081

10

Examples

Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

Each ATCC Deposit No:Z is contained in a plasmid vector. Table 7 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The following correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 7 as being isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

<u>Vector Used to Construct Library</u>	<u>Corresponding Deposited Plasmid</u>
Lambda Zap	pBluescript (pBS)
Uni-Zap XR	pBluescript (pBS)
Zap Express	pBK
lafmid BA	plafmid BA
pSport1	pSport1
pCMVSPORT 2.0	pCMVSPORT 2.0

25

pCMVSPORT 3.0

pCMVSPORT 3.0

pCR[®]2.1

pCR[®]2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., Nucleic Acids Res. 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS-. The S and K refers to the orientation of the polylinker to the T7 and T3 primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the fl origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the fl ori generates sense strand DNA and in the other, antisense.

Vectors pSPORT1, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993)). Vector lafmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR[®]2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al., Bio/Technology 9: (1991)). Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 7, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited by reference to Table 1A, Table 2, Table 6 and Table 7 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each ATCC Deposit No:Z.

TABLE 7

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HUKA HUKB HUKC HUKD HUKE HUKF HUKG	Human Uterine Cancer	Lambda ZAP II	LP01
HCNA HCNB	Human Colon	Lambda Zap II	LP01
HFFA	Human Fetal Brain, random primed	Lambda Zap II	LP01
HTWA	Resting T-Cell	Lambda ZAP II	LP01
HBQA	Early Stage Human Brain, random primed	Lambda ZAP II	LP01
HLMB HLMF HLMG HLMH HLMI HLMJ HLMM HLMN	breast lymph node CDNA library	Lambda ZAP II	LP01
HCQA HCQB	human colon cancer	Lambda ZAP II	LP01
HMEA HMEC HMED HMEE HMEF HMEG HMEI HMEJ HMEK HMEI	Human Microvascular Endothelial Cells, fract. A	Lambda ZAP II	LP01
HUSA HUSC	Human Umbilical Vein Endothelial Cells, fract. A	Lambda ZAP II	LP01
HLQA HLQB	Hepatocellular Tumor	Lambda ZAP II	LP01
HHGA HHGB HHGC HHGD	Hemangiopericytoma	Lambda ZAP II	LP01
HSDM	Human Striatum Depression, re-rescue	Lambda ZAP II	LP01
HUSH	H Umbilical Vein Endothelial Cells, frac A, re-excision	Lambda ZAP II	LP01
HSGS	Salivary gland, subtracted	Lambda ZAP II	LP01
HFXA HFXB HFXC HFXD HFXE HFXF HFXG HFXH	Brain frontal cortex	Lambda ZAP II	LP01
HPQA HPQB HPQC	PERM TF274	Lambda ZAP II	LP01
HFXJ HFXK	Brain Frontal Cortex, re-excision	Lambda ZAP II	LP01
HCWA HCWB HCWC HCWD HCWE HCWF HCWG HCWH HCWI HCWJ HCWK	CD34 positive cells (Cord Blood)	ZAP Express	LP02
HCUA HCUB HCUC	CD34 depleted Buffy Coat (Cord Blood)	ZAP Express	LP02
HRSM	A-14 cell line	ZAP Express	LP02
HRSA	A1-CELL LINE	ZAP Express	LP02
HCUD HCUE HCUF HCUG HCUH HCUI	CD34 depleted Buffy Coat (Cord Blood), re-excision	ZAP Express	LP02
HBXE HBXF HBXG	H. Whole Brain #2, re-excision	ZAP Express	LP02
HRLM	L8 cell line	ZAP Express	LP02
HBXA HBXB HBXC HBXD	Human Whole Brain #2 - Oligo dT > 1.5Kb	ZAP Express	LP02
HUDA HUDB HUDC	Testes	ZAP Express	LP02
HHTM HHTN HHTO	H. hypothalamus, frac A; re-excision	ZAP Express	LP02
HHTL	H. hypothalamus, frac A	ZAP Express	LP02
HASA HASD	Human Adult Spleen	Uni-ZAP XR	LP03
HFKC HFKD HFKE HFKF	Human Fetal Kidney	Uni-ZAP XR	LP03

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HFKG			
HE8A HE8B HE8C HE8D HE8E HE8F HE8M HE8N	Human 8 Week Whole Embryo	Uni-ZAP XR	LP03
HGBA HGBD HGBE HGBF HGBG HGBH HGBI	Human Gall Bladder	Uni-ZAP XR	LP03
HLHA HLHB HLHC HLHD HLHE HLHF HLHG HLHH HLHQ	Human Fetal Lung III	Uni-ZAP XR	LP03
HPMA HPMB HPMC HPMD HPME HPMF HPMG HPMH	Human Placenta	Uni-ZAP XR	LP03
HPRA HPRB HPRC HPRD	Human Prostate	Uni-ZAP XR	LP03
HSIA HSIC HSID HSIE	Human Adult Small Intestine	Uni-ZAP XR	LP03
HTEA HTEB HTEC HTED HTEE HTEF HTEG HTEH HTEI HTEJ HTEK	Human Testes	Uni-ZAP XR	LP03
HTPA HTPB HTPC HTPD HTPE	Human Pancreas Tumor	Uni-ZAP XR	LP03
HTTA HTTB HTTC HTTD HTTE HTTF	Human Testes Tumor	Uni-ZAP XR	LP03
HAPA HAPB HAPC HAPM	Human Adult Pulmonary	Uni-ZAP XR	LP03
HETA HETB HETC HETD HETE HETF HETG HETH HETI	Human Endometrial Tumor	Uni-ZAP XR	LP03
HHFB HHFC HHFD HHFE HHFF HHFG HHFH HHFI	Human Fetal Heart	Uni-ZAP XR	LP03
HHPB HHPD HHPD HHPE HHPF HHPG HHPH	Human Hippocampus	Uni-ZAP XR	LP03
HCE1 HCE2 HCE3 HCE4 HCE5 HCEB HCEC HCED HCEE HCEF HCEG	Human Cerebellum	Uni-ZAP XR	LP03
HUVB HUVB HUVD HUVE	Human Umbilical Vein, Endo. remake	Uni-ZAP XR	LP03
HSTA HSTB HSTC HSTD	Human Skin Tumor	Uni-ZAP XR	LP03
HTAA HTAB HTAC HTAD HTAE	Human Activated T-Cells	Uni-ZAP XR	LP03
HFEA HFEB HFEC	Human Fetal Epithelium (Skin)	Uni-ZAP XR	LP03
HJPA HJPB HJPC HJPD	HUMAN JURKAT MEMBRANE BOUND POLYSOMES	Uni-ZAP XR	LP03
HESA	Human epithelioid sarcoma	Uni-Zap XR	LP03
HLTA HLTB HLTC HLTD HLTE HLTF	Human T-Cell Lymphoma	Uni-ZAP XR	LP03
HFTA HFTB HFTC HFTD	Human Fetal Dura Mater	Uni-ZAP XR	LP03
HRDA HRDB HRDC HRDD HRDE HRDF	Human Rhabdomyosarcoma	Uni-ZAP XR	LP03
HCAA HCAB HCAC	Cem cells cyclohexamide treated	Uni-ZAP XR	LP03
HRGA HRGB HRGC HRGD	Raji Cells, cyclohexamide treated	Uni-ZAP XR	LP03
HSUA HSUB HSUC HSUM	Supt Cells, cyclohexamide treated	Uni-ZAP XR	LP03
HT4A HT4C HT4D	Activated T-Cells, 12 hrs.	Uni-ZAP XR	LP03
HE9A HE9B HE9C HE9D HE9E HE9F HE9G HE9H	Nine Week Old Early Stage Human	Uni-ZAP XR	LP03

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HE9M HE9N			
HATA HATB HATC HATD HATE	Human Adrenal Gland Tumor	Uni-ZAP XR	LP03
HT5A	Activated T-Cells, 24 hrs.	Uni-ZAP XR	LP03
HFGA HFGM	Human Fetal Brain	Uni-ZAP XR	LP03
HNEA HNEB HNEC HNED HNEE	Human Neutrophil	Uni-ZAP XR	LP03
HBGB HBGD	Human Primary Breast Cancer	Uni-ZAP XR	LP03
HBNA HBNB	Human Normal Breast	Uni-ZAP XR	LP03
HCAS	Cem Cells, cyclohexamide treated, subtra	Uni-ZAP XR	LP03
HHPS	Human Hippocampus, subtracted	pBS	LP03
HKCS HKCU	Human Colon Cancer, subtracted	pBS	LP03
HRGS	Raji cells, cyclohexamide treated, subtracted	pBS	LP03
HSUT	Supt cells, cyclohexamide treated, differentially expressed	pBS	LP03
HT4S	Activated T-Cells, 12 hrs, subtracted	Uni-ZAP XR	LP03
HCDA HCDB HCDC HCDD HCDE	Human Chondrosarcoma	Uni-ZAP XR	LP03
HOAA HOAB HOAC	Human Osteosarcoma	Uni-ZAP XR	LP03
HTLA HTLB HTLC HTLD HTLE HTLF	Human adult testis, large inserts	Uni-ZAP XR	LP03
HLMA HLMC HLMD	Breast Lymph node cDNA library	Uni-ZAP XR	LP03
H6EA H6EB H6EC	HL-60, PMA 4H	Uni-ZAP XR	LP03
HTXA HTXB HTXC HTXD HTXE HTXF HTXG HTXH	Activated T-Cell (12hs)/Thiouridine labelledEco	Uni-ZAP XR	LP03
HNFA HNFb HNFC HNFD HNFE HNFF HNFG HNFH HNFJ	Human Neutrophil, Activated	Uni-ZAP XR	LP03
HTOB HTOC	HUMAN TONSILS, FRACTION 2	Uni-ZAP XR	LP03
HMGB	Human OB MG63 control fraction I	Uni-ZAP XR	LP03
HOPB	Human OB HOS control fraction I	Uni-ZAP XR	LP03
HORB	Human OB HOS treated (10 nM E2) fraction I	Uni-ZAP XR	LP03
HSVA HSVB HSVC	Human Chronic Synovitis	Uni-ZAP XR	LP03
HROA	HUMAN STOMACH	Uni-ZAP XR	LP03
HBJA HBJB HBJC HBJD HBJE HBJF HBJG HBJH HBJI HBJJ HBJK	HUMAN B CELL LYMPHOMA	Uni-ZAP XR	LP03
HCRA HCRB HCRC	human corpus colosum	Uni-ZAP XR	LP03
HODA HODB HODC HODD	human ovarian cancer	Uni-ZAP XR	LP03
HDSA	Dermatofibrosarcoma Protuberance	Uni-ZAP XR	LP03
HMWA HMWB HMWC HMWD HMWE HMWF HMWG HMWH HMWI	Bone Marrow Cell Line (RS4;11)	Uni-ZAP XR	LP03

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HMWJ			
HSOA	stomach cancer (human)	Uni-ZAP XR	LP03
HERA	SKIN	Uni-ZAP XR	LP03
HMDA	Brain-medulloblastoma	Uni-ZAP XR	LP03
HGLA HGLB HGLD	Glioblastoma	Uni-ZAP XR	LP03
HEAA	H. Atrophic Endometrium	Uni-ZAP XR	LP03
HBCA HBCB	H. Lymph node breast Cancer	Uni-ZAP XR	LP03
HPWT	Human Prostate BPH, re-excision	Uni-ZAP XR	LP03
HFVG HFVH HFVI	Fetal Liver, subtraction II	pBS	LP03
HNFI	Human Neutrophils, Activated, re-excision	pBS	LP03
HBMB HBMC HBMD	Human Bone Marrow, re-excision	pBS	LP03
HKML HKMM HKMN	H. Kidney Medulla, re-excision	pBS	LP03
HKIX HKIY	H. Kidney Cortex, subtracted	pBS	LP03
HADT	H. Amygdala Depression, subtracted	pBS	LP03
H6AS	HL-60, untreated, subtracted	Uni-ZAP XR	LP03
H6ES	HL-60, PMA 4H, subtracted	Uni-ZAP XR	LP03
H6BS	HL-60, RA 4h, Subtracted	Uni-ZAP XR	LP03
H6CS	HL-60, PMA 1d, subtracted	Uni-ZAP XR	LP03
HTXJ HTXK	Activated T-cell(12h)/Thiouridine-re-excision	Uni-ZAP XR	LP03
HMSA HMSB HMSC HMSD HMSE HMSF HMSG HMSH HMSI HMSJ HMSK	Monocyte activated	Uni-ZAP XR	LP03
HAGA HAGB HAGC HAGD HAGE HAGF	Human Amygdala	Uni-ZAP XR	LP03
HSRA HSRB HSRE	STROMAL -OSTEOCLASTOMA	Uni-ZAP XR	LP03
HSRD HSRF HSRG HSRH	Human Osteoclastoma Stromal Cells - unamplified	Uni-ZAP XR	LP03
HSQA HSQB HSQC HSQD HSQE HSQF HSQG	Stromal cell TF274	Uni-ZAP XR	LP03
HSKA HSKB HSKC HSKD HSKE HSKF HSKZ	Smooth muscle, serum treated	Uni-ZAP XR	LP03
HSLA HSLB HSLC HSLD HSLE HSLF HSLG	Smooth muscle, control	Uni-ZAP XR	LP03
HSDA HSDD HSDE HSDF HSDG HSDH	Spinal cord	Uni-ZAP XR	LP03
HPWS	Prostate-BPH subtracted II	pBS	LP03
HSKW HSKX HSKY	Smooth Muscle- HASTE normalized	pBS	LP03
HFPB HFPC HFPD	H. Frontal cortex, epileptic; re-excision	Uni-ZAP XR	LP03
HSDI HSDJ HSDK	Spinal Cord, re-excision	Uni-ZAP XR	LP03
HSKN HSKO	Smooth Muscle Serum Treated, Norm	pBS	LP03
HSKG HSKH HSKI	Smooth muscle, serum induced, re-exc	pBS	LP03
HFCA HFCB HFCC HFCD HFCE HFCF	Human Fetal Brain	Uni-ZAP XR	LP04

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HBMV HBMW HBMX			
HOEA HOEB HOEC HOED HOEE HOEF HOEJ	Osteoblasts	Uni-ZAP XR	LP04
HAIA HAIB HAIC HAID HAIE HAIF	Epithelial-TNF α and INF induced	Uni-ZAP XR	LP04
HTGA HTGB HTGC HTGD	Apoptotic T-cell	Uni-ZAP XR	LP04
HMCA HMCB HMCC HMCD HMCE	Macrophage-oxLDL	Uni-ZAP XR	LP04
HMAA HMAB HMAC HMAD HMAE HMAF HMAG	Macrophage (GM-CSF treated)	Uni-ZAP XR	LP04
HPHA	Normal Prostate	Uni-ZAP XR	LP04
HPIA HPIB HPIC	LNCAP prostate cell line	Uni-ZAP XR	LP04
HPJA HPJB HPJC	PC3 Prostate cell line	Uni-ZAP XR	LP04
HOSE HOSF HOSG	Human Osteoclastoma, re-excision	Uni-ZAP XR	LP04
HTGE HTGF	Apoptotic T-cell, re-excision	Uni-ZAP XR	LP04
HMAJ HMAK	H Macrophage (GM-CSF treated), re-excision	Uni-ZAP XR	LP04
HACB HACC HACD	Human Adipose Tissue, re- excision	Uni-ZAP XR	LP04
HFAA	H. Frontal Cortex, Epileptic	Uni-ZAP XR	LP04
HFAA HFAB HFAC HFAD HFAE	Alzheimer's, spongy change	Uni-ZAP XR	LP04
HFAM	Frontal Lobe, Dementia	Uni-ZAP XR	LP04
HMIA HMIB HMIC	Human Manic Depression Tissue	Uni-ZAP XR	LP04
HTSA HTSE HTSF HTSG HTSH	Human Thymus	pBS	LP05
HPBA HPBB HPBC HPBD HPBE	Human Pineal Gland	pBS	LP05
HSAA HSAB HSAC	HSA 172 Cells	pBS	LP05
HSBA HSBB HSBC HSBM	HSC172 cells	pBS	LP05
HJAA HJAB HJAC HJAD	Jurkat T-cell G1 phase	pBS	LP05
HJBA HJBB HJBC HJBD	Jurkat T-Cell, S phase	pBS	LP05
HAFA HAFB	Aorta endothelial cells + TNF- α	pBS	LP05
HAWA HAWB HAWC	Human White Adipose	pBS	LP05
HTNA HTNB	Human Thyroid	pBS	LP05
HONA	Normal Ovary, Premenopausal	pBS	LP05
HARA HARB	Human Adult Retina	pBS	LP05
HLJA HLJB	Human Lung	pCMVSPORT 1	LP06
HOFM HOFN HOFO	H. Ovarian Tumor, II, OV5232	pCMVSPORT 2.0	LP07
HOGA HOGB HOGC	OV 10-3-95	pCMVSPORT 2.0	LP07
HCGL	CD34+cells, II	pCMVSPORT 2.0	LP07
HDLA	Hodgkin's Lymphoma I	pCMVSPORT 2.0	LP07
HDTA HDTB HDTC HDTD HDTE	Hodgkin's Lymphoma II	pCMVSPORT 2.0	LP07
HKAA HKAB HKAC HKAD HKAH HKAF HKAG HKAH	Keratinocyte	pCMVSPORT2.0	LP07
HCIM	CAPFINDER, Crohn's Disease, lib 2	pCMVSPORT 2.0	LP07

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HKAL	Keratinocyte, lib 2	pCMVSPORT2.0	LP07
HKAT	Keratinocyte, lib 3	pCMVSPORT2.0	LP07
HNDA	Nasal polyps	pCMVSPORT2.0	LP07
HDRA	H. Primary Dendritic Cells, lib 3	pCMVSPORT2.0	LP07
HOHA HOHB HOHC	Human Osteoblasts II,	pCMVSPORT2.0	LP07
HLDA HLDB HLDC	Liver, Hepatoma	pCMVSPORT3.0	LP08
HLDN HLDO HLDP	Human Liver, normal	pCMVSPORT3.0	LP08
HMTA	pBMC stimulated w/ poly I/C	pCMVSPORT3.0	LP08
HNTA	NTERA2, control	pCMVSPORT3.0	LP08
HDP A HDPB HDPC HDPD HDPF HDPG HDPH HDPI HDPJ HDPK	Primary Dendritic Cells, lib 1	pCMVSPORT3.0	LP08
HDPM HDPN HDPO HDPP	Primary Dendritic cells, frac 2	pCMVSPORT3.0	LP08
HMUA HMUB HMUC	Myeloid Progenitor Cell Line	pCMVSPORT3.0	LP08
HHEA HHEB HHEC HHED	T Cell helper I	pCMVSPORT3.0	LP08
HHEM HHEN HHEO HHEP	T cell helper II	pCMVSPORT3.0	LP08
HEQA HEQB HEQC	Human endometrial stromal cells	pCMVSPORT3.0	LP08
HJMA HJMB	Human endometrial stromal cells- treated with progesterone	pCMVSPORT3.0	LP08
HSWA HSWB HSWC	Human endometrial stromal cells- treated with estradiol	pCMVSPORT3.0	LP08
HSYA HSYB HSYC	Human Thymus Stromal Cells	pCMVSPORT3.0	LP08
HLWA HLWB HLWC	Human Placenta	pCMVSPORT3.0	LP08
HRAA HRAB HRAC	Rejected Kidney, lib 4	pCMVSPORT3.0	LP08
HMTM	PCR, pBMC I/C treated	PCR II	LP09
HMJA	H. Meningioma, M6	pSport 1	LP10
HMKA HMKB HMKC HMKD HMKE	H. Meningioma, M1	pSport 1	LP10
HUSG HUSI	Human umbilical vein endothelial cells, IL-4 induced	pSport 1	LP10
HUSX HUSY	Human Umbilical Vein Endothelial Cells, uninduced	pSport 1	LP10
HOFA	Ovarian Tumor I, OV5232	pSport 1	LP10
HCFA HCFB HCFC HCFD	T-Cell PHA 16 hrs	pSport 1	LP10
HCFL HCFM HCFN HCFO	T-Cell PHA 24 hrs	pSport 1	LP10
HADA HADC HADD HADE HADG	Human Adipose	pSport 1	LP10
HOVA HOVB HOVC	Human Ovary	pSport 1	LP10
HTWB HTWC HTWD HTWE HTWF	Resting T-Cell Library, II	pSport 1	LP10
HMMA	Spleen metastatic melanoma	pSport 1	LP10
HLYA HLYB HLYC HLYD HLYE	Spleen, Chronic lymphocytic leukemia	pSport 1	LP10
HCGA	CD34+ cell, I	pSport 1	LP10
HEOM HEON	Human Eosinophils	pSport 1	LP10
HTDA	Human Tonsil, Lib 3	pSport 1	LP10
HSPA	Salivary Gland, Lib 2	pSport 1	LP10
HCHA HCHB HCHC	Breast Cancer cell line, MDA 36	pSport 1	LP10
HCHM HCHN	Breast Cancer Cell line, angiogenic	pSport 1	LP10

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HCIA	Crohn's Disease	pSport 1	LP10
HDAA HDAB HDAC	HEL cell line	pSport 1	LP10
HABA	Human Astrocyte	pSport 1	LP10
HUFA HUFB HUFC	Ulcerative Colitis	pSport 1	LP10
HNTM	NTERA2 + retinoic acid, 14 days	pSport 1	LP10
HDQA	Primary Dendritic cells,CapFinder2, frac 1	pSport 1	LP10
HDQM	Primary Dendritic Cells, CapFinder, frac 2	pSport 1	LP10
HLDX	Human Liver, normal,CapFinder	pSport 1	LP10
HULA HULB HULC	Human Dermal Endothelial Cells,untreated	pSport1	LP10
HUMA	Human Dermal Endothelial cells,treated	pSport1	LP10
HCJA	Human Stromal Endometrial fibroblasts, untreated	pSport1	LP10
HCJM	Human Stromal endometrial fibroblasts, treated w/ estradiol	pSport1	LP10
HEDA	Human Stromal endometrial fibroblasts, treated with progesterone	pSport1	LP10
HFNA	Human ovary tumor cell OV350721	pSport1	LP10
HKGA HKGB HKGC HKGD	Merkel Cells	pSport1	LP10
HISA HISB HISC	Pancreas Islet Cell Tumor	pSport1	LP10
HLSA	Skin, burned	pSport1	LP10
HBZA	Prostate,BPH, Lib 2	pSport 1	LP10
HBZS	Prostate BPH,Lib 2, subtracted	pSport 1	LP10
HFIA HFIB HFIC	Synovial Fibroblasts (control)	pSport 1	LP10
HFIH HFII HFIJ	Synovial hypoxia	pSport 1	LP10
HFIT HFIU HFIV	Synovial IL-1/TNF stimulated	pSport 1	LP10
HGCA	Messangial cell, frac 1	pSport1	LP10
HMVA HMOV HMVC	Bone Marrow Stromal Cell, untreated	pSport1	LP10
HFIX HFIY HFIZ	Synovial Fibroblasts (II1/TNF), subt	pSport1	LP10
HFOX HFOY HFOZ	Synovial hypoxia-RSF subtracted	pSport1	LP10
HMQA HMQB HMQC HMQD	Human Activated Monocytes	Uni-ZAP XR	LP11
HLIA HLIB HLIC	Human Liver	pCMVSPORT 1	LP012
HHBA HHBB HHBC HHBD HHBE	Human Heart	pCMVSPORT 1	LP012
HBBA HBBB	Human Brain	pCMVSPORT 1	LP012
HLJA HLJB HLJC HLJD HLJE	Human Lung	pCMVSPORT 1	LP012
HOGA HOGB HOGC	Ovarian Tumor	pCMVSPORT 2.0	LP012
HTJM	Human Tonsils, Lib 2	pCMVSPORT 2.0	LP012
HAMF HAMG	KMH2	pCMVSPORT 3.0	LP012
HAJA HAJB HAJC	L428	pCMVSPORT 3.0	LP012
HWBA HWBB HWBC	Dendritic cells, pooled	pCMVSPORT 3.0	LP012

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HWBD HWBE			
HWAA HWAB HWAC HWAD HWAE	Human Bone Marrow, treated	pCMVSPORT 3.0	LP012
HYAA HYAB HYAC	B Cell lymphoma	pCMVSPORT 3.0	LP012
HWHG HWHH HWHI	Healing groin wound, 6.5 hours post incision	pCMVSPORT 3.0	LP012
HWHP HWHQ HWHR	Healing groin wound; 7.5 hours post incision	pCMVSPORT 3.0	LP012
HARM	Healing groin wound - zero hr post-incision (control)	pCMVSPORT 3.0	LP012
HBIM	Olfactory epithelium; nasalcavity	pCMVSPORT 3.0	LP012
HWDA	Healing Abdomen wound; 70&90 min post incision	pCMVSPORT 3.0	LP012
HWEA	Healing Abdomen Wound;15 days post incision	pCMVSPORT 3.0	LP012
HWJA	Healing Abdomen Wound;21&29 days	pCMVSPORT 3.0	LP012
HNAL	Human Tongue, frac 2	pSport1	LP012
HMJA	H. Meningioma, M6	pSport1	LP012
HMKA HMKB HMKC HMKD HMKE	H. Meningioma, M1	pSport1	LP012
HOFA	Ovarian Tumor I, OV5232	pSport1	LP012
HCFA HCFB HCFC HCFO	T-Cell PHA 16 hrs	pSport1	LP012
HCFL HCFM HCFN HCFO	T-Cell PHA 24 hrs	pSport1	LP012
HMMA HMMB HMMC	Spleen metastatic melanoma	pSport1	LP012
HTDA	Human Tonsil, Lib 3	pSport1	LP012
HDBA	Human Fetal Thymus	pSport1	LP012
HDUA	Pericardium	pSport1	LP012
HBZA	Prostate,BPH, Lib 2	pSport1	LP012
HWCA	Larynx tumor	pSport1	LP012
HWKA	Normal lung	pSport1	LP012
HSMB	Bone marrow stroma,treated	pSport1	LP012
HBHM	Normal trachea	pSport1	LP012
HLFC	Human Larynx	pSport1	LP012
HLRB	Siebben Polyposis	pSport1	LP012
HNIA	Mammary Gland	pSport1	LP012
HNJB	Palate carcinoma	pSport1	LP012
HNKA	Palate normal	pSport1	LP012
HMZA	Pharynx carcinoma	pSport1	LP012
HABG	Cheek Carcinoma	pSport1	LP012
HMZM	Pharynx Carcinoma	pSport1	LP012
HDRM	Larynx Carcinoma	pSport1	LP012
HVAA	Pancreas normal PCA4 No	pSport1	LP012
HICA	Tongue carcinoma	pSport1	LP012
HUKA HUKB HUKC HUKD HUKE	Human Uterine Cancer	Lambda ZAP II	LP013
HFFA	Human Fetal Brain, random primed	Lambda ZAP II	LP013
HTUA	Activated T-cell labeled with 4- thioluri	Lambda ZAP II	LP013

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HBQA	Early Stage Human Brain, random primed	Lambda ZAP II	LP013
HMEB	Human microvascular Endothelial cells, fract. B	Lambda ZAP II	LP013
HUSH	Human Umbilical Vein Endothelial cells, fract. A, re-excision	Lambda ZAP II	LP013
HLQC HLQD	Hepatocellular tumor, re-excision	Lambda ZAP II	LP013
HTWJ HTWK HTWL	Resting T-cell, re-excision	Lambda ZAP II	LP013
HF6S	Human Whole 6 week Old Embryo (II), subt	pBluescript	LP013
HHPS	Human Hippocampus, subtracted	pBluescript	LP013
HL1S	LNCAP, differential expression	pBluescript	LP013
HLHS HLHT	Early Stage Human Lung, Subtracted	pBluescript	LP013
HSUS	Supt cells, cyclohexamide treated, subtracted	pBluescript	LP013
HSUT	Supt cells, cyclohexamide treated, differentially expressed	pBluescript	LP013
HSDS	H. Striatum Depression, subtracted	pBluescript	LP013
HPTZ	Human Pituitary, Subtracted VII	pBluescript	LP013
HSDX	H. Striatum Depression, subt II	pBluescript	LP013
HSDZ	H. Striatum Depression, subt	pBluescript	LP013
HPBA HPBB HPBC HPBD HPBE	Human Pineal Gland	pBluescript SK-	LP013
HRTA	Colorectal Tumor	pBluescript SK-	LP013
HSBA HSBB HSBC HSBM	HSC172 cells	pBluescript SK-	LP013
HJAA HJAB HJAC HJAD	Jurkat T-cell G1 phase	pBluescript SK-	LP013
HJBA HJBB HJBC HJBD	Jurkat T-cell, S1 phase	pBluescript SK-	LP013
HTNA HTNB	Human Thyroid	pBluescript SK-	LP013
HAHA HAHB	Human Adult Heart	Uni-ZAP XR	LP013
HE6A	Whole 6 week Old Embryo	Uni-ZAP XR	LP013
HFCA HFCE HFCC HFCD HFCE	Human Fetal Brain	Uni-ZAP XR	LP013
HFKC HFKD HFKE HFKF HFKG	Human Fetal Kidney	Uni-ZAP XR	LP013
HGBA HGBD HGBE HGBF HGBG	Human Gall Bladder	Uni-ZAP XR	LP013
HPRA HPRB HPRC HPRD	Human Prostate	Uni-ZAP XR	LP013
HTEA HTEB HTEC HTED HTEE	Human Testes	Uni-ZAP XR	LP013
HTTA HTTB HTTC HTTD HTTE	Human Testes Tumor	Uni-ZAP XR	LP013
HYBA HYBB	Human Fetal Bone	Uni-ZAP XR	LP013
HFLA	Human Fetal Liver	Uni-ZAP XR	LP013
HHFB HHFC HHFD HHFE HHFF	Human Fetal Heart	Uni-ZAP XR	LP013
HUVB HUV C HUVD HUVE	Human Umbilical Vein, End. remake	Uni-ZAP XR	LP013
HTHB HTHC HTHD	Human Thymus	Uni-ZAP XR	LP013
HSTA HSTB HSTC HSTD	Human Skin Tumor	Uni-ZAP XR	LP013

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HTAA HTAB HTAC HTAD HTAE	Human Activated T-cells	Uni-ZAP XR	LP013
HFEA HFEB HFEC	Human Fetal Epithelium (skin)	Uni-ZAP XR	LP013
HJPA HJPB HJPC HJPD	Human Jurkat Membrane Bound Polysomes	Uni-ZAP XR	LP013
HESA	Human Epithelioid Sarcoma	Uni-ZAP XR	LP013
HALS	Human Adult Liver, Subtracted	Uni-ZAP XR	LP013
HFTA HFTB HFTC HFTD	Human Fetal Dura Mater	Uni-ZAP XR	LP013
HCAA HCAB HCAC	Cem cells, cyclohexamide treated	Uni-ZAP XR	LP013
HRGA HRGB HRGC HRGD	Raji Cells, cyclohexamide treated	Uni-ZAP XR	LP013
HE9A HE9B HE9C HE9D HE9E	Nine Week Old Early Stage Human	Uni-ZAP XR	LP013
HSFA	Human Fibrosarcoma	Uni-ZAP XR	LP013
HATA HATB HATC HATD HATE	Human Adrenal Gland Tumor	Uni-ZAP XR	LP013
HTRA	Human Trachea Tumor	Uni-ZAP XR	LP013
HE2A HE2D HE2E HE2H HE2I	12 Week Old Early Stage Human	Uni-ZAP XR	LP013
HE2B HE2C HE2F HE2G HE2P	12 Week Old Early Stage Human, II	Uni-ZAP XR	LP013
HNEA HNEB HNEC HNED HNEE	Human Neutrophil	Uni-ZAP XR	LP013
HBGA	Human Primary Breast Cancer	Uni-ZAP XR	LP013
HPTS HPTT HPTU	Human Pituitary, subtracted	Uni-ZAP XR	LP013
HMQA HMQB HMQC HMQD	Human Activated Monocytes	Uni-ZAP XR	LP013
HOAA HOAB HOAC	Human Osteosarcoma	Uni-ZAP XR	LP013
HTOA HTOD HTOE HTOF HTOG	human tonsils	Uni-ZAP XR	LP013
HMGB	Human OB MG63 control fraction I	Uni-ZAP XR	LP013
HOPB	Human OB HOS control fraction I	Uni-ZAP XR	LP013
HOQB	Human OB HOS treated (1 nM E2) fraction I	Uni-ZAP XR	LP013
HAUA HAUB HAUC	Amniotic Cells - TNF induced	Uni-ZAP XR	LP013
HAQA HAQB HAQC HAQD	Amniotic Cells - Primary Culture	Uni-ZAP XR	LP013
HROA HROC	HUMAN STOMACH	Uni-ZAP XR	LP013
HBJA HBJB HBJC HBJD HBJE	HUMAN B CELL LYMPHOMA	Uni-ZAP XR	LP013
HODA HODB HODC HODD	human ovarian cancer	Uni-ZAP XR	LP013
HCPA	Corpus Callosum	Uni-ZAP XR	LP013
HSOA	stomach cancer (human)	Uni-ZAP XR	LP013
HERA	SKIN	Uni-ZAP XR	LP013
HMDA	Brain-medulloblastoma	Uni-ZAP XR	LP013
HGLA HGLB HGLD	Glioblastoma	Uni-ZAP XR	LP013
HWTA HWTB HWTC	wilm's tumor	Uni-ZAP XR	LP013
HEAA	H. Atrophic Endometrium	Uni-ZAP XR	LP013
HAPN HAPO HAPP HAPQ HAPR	Human Adult Pulmonary;re-excision	Uni-ZAP XR	LP013
HLTG HLTH	Human T-cell lymphoma;re-	Uni-ZAP XR	LP013

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
	excision		
HAHC HAHD HAHE	Human Adult Heart;re-excision	Uni-ZAP XR	LP013
HAGA HAGB HAGC HAGD HAGE	Human Amygdala	Uni-ZAP XR	LP013
HSJA HSJB HSJC	Smooth muscle-ILb induced	Uni-ZAP XR	LP013
HSJA HSHB HSHC	Smooth muscle, IL1b induced	Uni-ZAP XR	LP013
HPWA HPWB HPWC HPWD HPWE	Prostate BPH	Uni-ZAP XR	LP013
HPIA HPIB HPIC	LNCAP prostate cell line	Uni-ZAP XR	LP013
HPJA HPJB HPJC	PC3 Prostate cell line	Uni-ZAP XR	LP013
HBTA	Bone Marrow Stroma, TNF&LPS ind	Uni-ZAP XR	LP013
HMCF HMCB HMCH HMCJ HMCJ	Macrophage-oxLDL; re-excision	Uni-ZAP XR	LP013
HAGG HAGH HAGI	Human Amygdala;re-excision	Uni-ZAP XR	LP013
HACA	H. Adipose Tissue	Uni-ZAP XR	LP013
HKFB	K562 + PMA (36 hrs),re-excision	ZAP Express	LP013
HCWT HCWU HCWV	CD34 positive cells (cord blood),re-ex	ZAP Express	LP013
HBWA	Whole brain	ZAP Express	LP013
HBXA HBXB HBXC HBXD	Human Whole Brain #2 - Oligo dT > 1.5Kb	ZAP Express	LP013
HAVM	Temporal cortex-Alzheimer	pT-Adv	LP014
HAVT	Hippocampus, Alzheimer Subtracted	pT-Adv	LP014
HHAS	CHME Cell Line	Uni-ZAP XR	LP014
HAJR	Larynx normal	pSport 1	LP014
HWLE HWLF HWLG HWLH	Colon Normal	pSport 1	LP014
HCRM HCRN HCRO	Colon Carcinoma	pSport 1	LP014
HWLI HWLJ HWLK	Colon Normal	pSport 1	LP014
HWLQ HWLR HWLS HWLT	Colon Tumor	pSport 1	LP014
HBFM	Gastrocnemius Muscle	pSport 1	LP014
HBOD HBOE	Quadriceps Muscle	pSport 1	LP014
HBKD HBKE	Soleus Muscle	pSport 1	LP014
HCCM	Pancreatic Langerhans	pSport 1	LP014
HWGA	Larynx carcinoma	pSport 1	LP014
HWGM HWGN	Larynx carcinoma	pSport 1	LP014
HWLA HWLB HWLC	Normal colon	pSport 1	LP014
HWLM HWLN	Colon Tumor	pSport 1	LP014
HVAM HVAN HVAO	Pancreas Tumor	pSport 1	LP014
HWGQ	Larynx carcinoma	pSport 1	LP014
HAQM HAQN	Salivary Gland	pSport 1	LP014
HASM	Stomach; normal	pSport 1	LP014
HBCM	Uterus; normal	pSport 1	LP014
HCDM	Testis; normal	pSport 1	LP014
HDJM	Brain; normal	pSport 1	LP014
HEFM	Adrenal Gland,normal	pSport 1	LP014
HBAA	Rectum normal	pSport 1	LP014

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HFDM	Rectum tumour	pSport 1	LP014
HGAM	Colon, normal	pSport 1	LP014
HHMM	Colon, tumour	pSport 1	LP014
HCLB HCLC	Human Lung Cancer	Lambda Zap II	LP015
HRLA	L1 Cell line	ZAP Express	LP015
HHAM	Hypothalamus, Alzheimer's	pCMVSport 3.0	LP015
HKBA	Ku 812F Basophils Line	pSport 1	LP015
HS2S	Saos2, Dexamethosome Treated	pSport 1	LP016
HA5A	Lung Carcinoma A549 TNFalpha activated	pSport 1	LP016
HTFM	TF-1 Cell Line GM-CSF Treated	pSport 1	LP016
HYAS	Thyroid Tumour	pSport 1	LP016
HUTS	Larynx Normal	pSport 1	LP016
HXOA	Larynx Tumor	pSport 1	LP016
HEAH	Ea.hy.926 cell line	pSport 1	LP016
HINA	Adenocarcinoma Human	pSport 1	LP016
HRMA	Lung Mesothelium	pSport 1	LP016
HLCL	Human Pre-Differentiated Adipocytes	Uni-Zap XR	LP017
HS2A	Saos2 Cells	pSport 1	LP020
HS2I	Saos2 Cells; Vitamin D3 Treated	pSport 1	LP020
HUCM	CHME Cell Line, untreated	pSport 1	LP020
HEPN	Aryepiglottis Normal	pSport 1	LP020
HPSN	Sinus Piniformis Tumour	pSport 1	LP020
HNSA	Stomach Normal	pSport 1	LP020
HNSM	Stomach Tumour	pSport 1	LP020
HNLA	Liver Normal Met5No	pSport 1	LP020
HUTA	Liver Tumour Met 5 Tu	pSport 1	LP020
HOCN	Colon Normal	pSport 1	LP020
HOCT	Colon Tumor	pSport 1	LP020
HTNT	Tongue Tumour	pSport 1	LP020
HLXN	Larynx Normal	pSport 1	LP020
HLXT	Larynx Tumour	pSport 1	LP020
HTYN	Thymus	pSport 1	LP020
HPLN	Placenta	pSport 1	LP020
HTNG	Tongue Normal	pSport 1	LP020
HZAA	Thyroid Normal (SDCA2 No)	pSport 1	LP020
HWES	Thyroid Thyroiditis	pSport 1	LP020
HFHD	Ficolled Human Stromal Cells, 5Fu treated	pTrip1Ex2	LP021
HFHM,HFHN	Ficolled Human Stromal Cells, Untreated	pTrip1Ex2	LP021
HPCI	Hep G2 Cells, lambda library	lambda Zap-CMV XR	LP021
HBCA,HBCB,HBCC	H. Lymph node breast Cancer	Uni-ZAP XR	LP021
HCOK	Chondrocytes	pSPORT1	LP022
HDCA, HDCB, HDCC	Dendritic Cells From CD34 Cells	pSPORT1	LP022
HDMA, HDMB	CD40 activated monocyte dendritic cells	pSPORT1	LP022
HDDM, HDDN, HDDO	LPS activated derived dendritic	pSPORT1	LP022

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
	cells		
HPCR	Hep G2 Cells, PCR library	lambda Zap-CMV XR	LP022
HAAA, HAAB, HAAC	Lung, Cancer (4005313A3): Invasive Poorly Differentiated Lung Adenocarcinoma	pSPORT1	LP022
HIPA, HIPB, HIPC	Lung, Cancer (4005163 B7): Invasive, Poorly Diff. Adenocarcinoma, Metastatic	pSPORT1	LP022
HOOH, HOOI	Ovary, Cancer: (4004562 B6) Papillary Serous Cystic Neoplasm, Low Malignant Pot	pSPORT1	LP022
HIDA	Lung, Normal: (4005313 B1)	pSPORT1	LP022
HUJA,HUJB,HUJC,HUJD,HUJE	B-Cells	pCMVSport 3.0	LP022
HNOA,HNOB,HNOC,HNOD	Ovary, Normal: (9805C040R)	pSPORT1	LP022
HNLM	Lung, Normal: (4005313 B1)	pSPORT1	LP022
HSCL	Stromal Cells	pSPORT1	LP022
HAAX	Lung, Cancer: (4005313 A3) Invasive Poorly-differentiated Metastatic lung adenocarcinoma	pSPORT1	LP022
HUUA,HUUB,HUUC,HUUD	B-cells (unstimulated)	pTrip1Ex2	LP022
HWWA,HWWB,HWWC,HWWD,HWWE,HWWF,HWWG	B-cells (stimulated)	pSPORT1	LP022
HCCC	Colon, Cancer: (9808C064R)	pCMVSport 3.0	LP023
HPDO HPDP HPDQ HPDR HPD	Ovary, Cancer (9809C332): Poorly differentiated adenocarcinoma	pSport 1	LP023
HPCO HPCP HPCQ HPCT	Ovary, Cancer (15395A1F): Grade II Papillary Carcinoma	pSport 1	LP023
HOCM HOCO HOCQ HOCQ	Ovary, Cancer: (15799A1F) Poorly differentiated carcinoma	pSport 1	LP023
HCBM HCBN HCBO	Breast, Cancer: (4004943 A5)	pSport 1	LP023
HNBT HNBU HNBV	Breast, Normal: (4005522B2)	pSport 1	LP023
HBCP HBCQ	Breast, Cancer: (4005522 A2)	pSport 1	LP023
HBCJ	Breast, Cancer: (9806C012R)	pSport 1	LP023
HSAM HSAN	Stromal cells 3.88	pSport 1	LP023
HVCA HVCB HVCC HVCD	Ovary, Cancer: (4004332 A2)	pSport 1	LP023
HSCK HSEN HSEO	Stromal cells (HBM3.18)	pSport 1	LP023
HSCP HSCQ	stromal cell clone 2.5	pSport 1	LP023
HUXA	Breast Cancer: (4005385 A2)	pSport 1	LP023
HCOM HCON HCOO HCOP HCOQ	Ovary, Cancer (4004650 A3): Well-Differentiated Micropapillary Serous Carcinoma	pSport 1	LP023
HBNM	Breast, Cancer: (9802C020E)	pSport 1	LP023
HVVA HVVB HVVC HVVD HVVE	Human Bone Marrow, treated	pSport 1	LP023

Two nonlimiting examples are provided below for isolating a particular clone from the deposited sample of plasmid cDNAs cited for that clone in Table 7. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to the nucleotide sequence of SEQ ID NO:X.

5 Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with ^{32}P - γ -ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring, NY (1982)). The plasmid mixture is transformed into a suitable host,
10 as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening
15 (e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the nucleotide sequence of SEQ ID NO:X are synthesized and used to amplify the desired cDNA using the
20 deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 μl of reaction mixture with 0.5 μg of the above cDNA template. A convenient reaction mixture is 1.5-5 mM MgCl_2 , 0.01% (w/v) gelatin, 20 μM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five
25 cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a
30 gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., *Nucleic Acids Res.* 21(7):1683-1684 (1993)).

35 Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer

specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the sequence corresponding to SEQ ID NO:X according to the method described in Example 1. (See also, Sambrook.)

Example 3: Tissue specific expression analysis

The Human Genome Sciences, Inc. (HGS) database is derived from sequencing tissue and/or disease specific cDNA libraries. Libraries generated from a particular tissue are selected and the specific tissue expression pattern of EST groups or assembled contigs within these libraries is determined by comparison of the expression patterns of those groups or contigs within the entire database. ESTs and assembled contigs which show tissue specific expression are selected.

The original clone from which the specific EST sequence was generated, or in the case of an assembled contig, the clone from which the 5' most EST sequence was generated, is obtained from the catalogued library of clones and the insert amplified by PCR using methods known in the art. The PCR product is denatured and then transferred in 96 or 384 well format to a nylon membrane (Schleicher and Scheull) generating an array filter of tissue specific clones. Housekeeping genes, maize genes, and known tissue specific genes are included on the filters. These targets can be used in signal normalization and to validate assay sensitivity. Additional targets are included to monitor probe length and specificity of hybridization.

Radioactively labeled hybridization probes are generated by first strand cDNA synthesis

per the manufacturer's instructions (Life Technologies) from mRNA/RNA samples prepared from the specific tissue being analyzed (e.g., prostate, prostate cancer, ovarian, ovarian cancer, etc.). The hybridization probes are purified by gel exclusion chromatography, quantitated, and hybridized with the array filters in hybridization bottles at 65°C overnight. The filters are washed under stringent conditions and signals are captured using a Fuji phosphorimager.

Data is extracted using AIS software and following background subtraction, signal normalization is performed. This includes a normalization of filter-wide expression levels between different experimental runs. Genes that are differentially expressed in the tissue of interest are identified.

Example 4: Chromosomal Mapping of the Polynucleotides

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions: 30 seconds, 95°C; 1 minute, 56°C; 1 minute, 70°C. This cycle is repeated 32 times followed by one 5 minute cycle at 70°C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions are analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

Example 5: Bacterial Expression of a Polypeptide

A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates

and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalactopyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4°C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8. The column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4°C or frozen at -80°C.

In addition to the above expression vector, the present invention further includes an expression vector, called pHE4a (ATCC Accession Number 209645, deposited on February 25, 1998) which contains phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lacIq). The origin of replication (oriC) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter and operator sequences are made synthetically.

DNA can be inserted into the pHE4a by restricting the vector with NdeI and XbaI,

BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

Example 6: Purification of a Polypeptide from an Inclusion Body

The following alternative method can be used to purify a polypeptide expressed in *E coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10°C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 µm membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM

sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant A_{280} monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 μ g of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

Example 7: Cloning and Expression of a Polypeptide in a Baculovirus Expression System

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the Autographa californica nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from E. coli under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., Virology 170:31-39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon, is amplified using the PCR protocol described in Example 1. If a naturally occurring signal sequence is used to produce the polypeptide of the present invention, the pA2

vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

5 The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art.

10 The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. E. coli HB101 or other suitable E. coli hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria
15 containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five µg of a plasmid containing the polynucleotide is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA,
20 Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One µg of BaculoGold™ virus DNA and 5 µg of the plasmid are mixed in a sterile well of a microtiter plate containing 50 µl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 µl Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the
25 transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27° C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27° C for four days.

30 After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, supra. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life
35 Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 µl of Grace's medium and the

suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4° C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 µCi of ³⁵S-methionine and 5 µCi ³⁵S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

Example 8: Expression of a Polypeptide in Mammalian Cells

The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as DHFR, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry

several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991)). Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No.209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If a naturally occurring signal sequence is used to produce the polypeptide of the present invention, the vector does not need a second signal peptide. Alternatively, if a naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., International Publication No. WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five μ g of the expression plasmid pC6 or pC4 is cotransfected with 0.5 μ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1

mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μ M, 2 μ M, 5 μ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 μ M. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Example 9: Protein Fusions

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988)). Similarly, fusion to IgG-1, IgG-3, and albumin increases the halflife time *in vivo*. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (ATCC Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the polypeptide of the present invention, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., International Publication No. WO 96/34891.)

Human IgG Fc region:

GGGATCCGGAGCCCAAATCTTCTGACAAAACCTCACACATGCCCACCGTGCCCAGCAC
CTGAATTCGAGGGTGCACCGTCAGTCTTCTTCCCCCAAAACCCAAGGACACCCT
5 CATGATCTCCCGGACTCCTGAGGTCACATGCGTGGTGGTGGACGTAAGCCACGAAGA
CCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC
AAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGT
CCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC
CCTCCCAACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACC
10 ACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCT
GACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGTGGAGTGGGAGAGCAA
TGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTC
CTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGT
CTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC
15 TCCCTGTCTCCGGGTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO: 1)

Example 10: Production of an Antibody from a Polypeptide

a) Hybridoma Technology

The antibodies of the present invention can be prepared by a variety of methods. (See,
20 Current Protocols, Chapter 2.) As one example of such methods, cells expressing a polypeptide of
the present invention are administered to an animal to induce the production of sera containing
polyclonal antibodies. In a preferred method, a preparation of a polypeptide of the present
invention is prepared and purified to render it substantially free of natural contaminants. Such a
preparation is then introduced into an animal in order to produce polyclonal antisera of greater
25 specific activity.

Monoclonal antibodies specific for a polypeptide of the present invention are prepared
using hybridoma technology (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol.
6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal
Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal
30 (preferably a mouse) is immunized with a polypeptide of the present invention or, more preferably,
with a secreted polypeptide-expressing cell. Such polypeptide-expressing cells are cultured in any
suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with
10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of
nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

35 The splenocytes of such mice are extracted and fused with a suitable myeloma cell line.
Any suitable myeloma cell line may be employed in accordance with the present invention;
however, it is preferable to employ the parent myeloma cell line (SP2O), available from the

ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide of the present invention .

Alternatively, additional antibodies capable of binding to a polypeptide of the present invention can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the polypeptide-specific antibody can be blocked by said polypeptide. Such antibodies comprise anti-idiotypic antibodies to the polypeptide-specific antibody and are used to immunize an animal to induce formation of further polypeptide-specific antibodies.

For *in vivo* use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., International Publication No. WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985)).

b) Isolation Of Antibody Fragments Directed Against a Polypeptide of the Present Invention From A Library Of scFvs

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against a polypeptide of the present invention to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in International Publication No. WO 92/01047. To rescue phage displaying antibody fragments, approximately 10^9 *E. coli* harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2×10^8 TU of delta gene 3 helper (M13 delta gene III, see International Publication No. WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet

resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 ug/ml kanamycin and grown overnight. Phage are prepared as described in International Publication No. WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 µm filter (Minisart NML; Sartorius) to give a final concentration of approximately 10^{13} transducing units/ml (ampicillin-resistant clones).

Panning of the Library. Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 µg/ml or 10 µg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 10^{13} TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 µg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 µg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., International Publication No. WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

Example 11: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide

RNA isolated from entire families or individual patients presenting with an allergic and/or asthmatic disease or disorder is isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X; and/or the nucleotide sequence of the cDNA contained in ATCC Deposit No:Z. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase (Epicentre Technologies). The intron-exon boundaries of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations are then cloned and sequenced to validate the results of the direct sequencing.

PCR products are cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenin deoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991)). Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

Example 12: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbound polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbound conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

Example 13: Formulation

The invention also provides methods of preventing, treating and/or ameliorating an allergic and/or asthmatic disease or disorder by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the Therapeutic is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An

intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Therapeutics can be administered orally, rectally, parenterally, intracistemally, 5 intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection 10 and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" 15 refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. 20 Suitable examples of sustained-release Therapeutics include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

25 Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

30 In a preferred embodiment, polypeptide, polynucleotide, and antibody compositions of the invention are formulated in a biodegradable, polymeric drug delivery system, for example as described in U.S. Patent Nos. 4,938,763; 5,278,201; 5,278,202; 5,324,519; 5,340,849; and 5,487,897 and in International Publication Numbers WO01/35929, WO00/24374, and WO00/06117 which are hereby incorporated by reference in their entirety. In specific preferred 35 embodiments the polypeptide, polynucleotide, and antibody compositions of the invention are formulated using the ATRIGEL® Biodegradable System of Atrix Laboratories, Inc. (Fort Collins, Colorado).

Examples of biodegradable polymers which can be used in the formulation of polypeptide, polynucleotide, and antibody compositions, include but are not limited to, polylactides, polyglycolides, polycaprolactones, polyanhydrides, polyamides, polyurethanes, polyesteramides, polyorthoesters, polydioxanones, polyacetals, polyketals, polycarbonates, polyorthocarbonates, polyphosphazenes, polyhydroxybutyrates, polyhydroxyvalerates, polyalkylene oxalates, polyalkylene succinates, poly(malic acid), poly(amino acids), poly(methyl vinyl ether), poly(maleic anhydride), polyvinylpyrrolidone, polyethylene glycol, polyhydroxycellulose, chitin, chitosan, and copolymers, terpolymers, or combinations or mixtures of the above materials. The preferred polymers are those that have a lower degree of crystallization and are more hydrophobic.

These polymers and copolymers are more soluble in the biocompatible solvents than the highly crystalline polymers such as polyglycolide and chitin which also have a high degree of hydrogen-bonding. Preferred materials with the desired solubility parameters are the polylactides, polycaprolactones, and copolymers of these with glycolide in which there are more amorphous regions to enhance solubility. In specific preferred embodiments, the biodegradable polymers which can be used in the formulation of polypeptide, polynucleotide, and antibody compositions are poly(lactide-co-glycolides). Polymer properties such as molecular weight, hydrophobicity, and lactide/glycolide ratio may be modified to obtain the desired polypeptide, polynucleotide, or antibody release profile (See, e.g., Ravivarapu et al., *Journal of Pharmaceutical Sciences* 89:732-741 (2000), which is hereby incorporated by reference in its entirety).

It is also preferred that the solvent for the biodegradable polymer be non-toxic, water miscible, and otherwise biocompatible. Examples of such solvents include, but are not limited to, N-methyl-2-pyrrolidone, 2-pyrrolidone, C2 to C6 alkanols, C1 to C15 alcohols, diols, triols, and tetraols such as ethanol, glycerine propylene glycol, butanol; C3 to C15 alkyl ketones such as acetone, diethyl ketone and methyl ethyl ketone; C3 to C15 esters such as methyl acetate, ethyl acetate, ethyl lactate; alkyl ketones such as methyl ethyl ketone, C1 to C15 amides such as dimethylformamide, dimethylacetamide and caprolactam; C3 to C20 ethers such as tetrahydrofuran, or solketal; tweens, triacetin, propylene carbonate, decylmethylsulfoxide, dimethyl sulfoxide, oleic acid, 1-dodecylazacycloheptan-2-one, Other preferred solvents are benzyl alcohol, benzyl benzoate, dipropylene glycol, tributyrin, ethyl oleate, glycerin, glycofural, isopropyl myristate, isopropyl palmitate, oleic acid, polyethylene glycol, propylene carbonate, and triethyl citrate. The most preferred solvents are N-methyl-2-pyrrolidone, 2-pyrrolidone, dimethyl sulfoxide, triacetin, and propylene carbonate because of the solvating ability and their compatibility.

Additionally, formulations comprising polypeptide, polynucleotide, and antibody compositions and a biodegradable polymer may also include release-rate modification agents and/or pore-forming agents. Examples of release-rate modification agents include, but are not limited to, fatty acids, triglycerides, other like hydrophobic compounds, organic solvents,

plasticizing compounds and hydrophilic compounds. Suitable release rate modification agents include, for example, esters of mono-, di-, and tricarboxylic acids, such as 2-ethoxyethyl acetate, methyl acetate, ethyl acetate, diethyl phthalate, dimethyl phthalate, dibutyl phthalate, dimethyl adipate, dimethyl succinate, dimethyl oxalate, dimethyl citrate, triethyl citrate, acetyl tributyl citrate, acetyl triethyl citrate, glycerol triacetate, di(n-butyl) sebecate, and the like; polyhydroxy alcohols, such as propylene glycol, polyethylene glycol, glycerin, sorbitol, and the like; fatty acids; triesters of glycerol, such as triglycerides, epoxidized soybean oil, and other epoxidized vegetable oils; sterols, such as cholesterol; alcohols, such as C.sub.6 -C.sub.12 alkanols, 2-ethoxyethanol. The release rate modification agent may be used singly or in combination with other such agents.

10 Suitable combinations of release rate modification agents include, but are not limited to, glycerin/propylene glycol, sorbitol/glycerine, ethylene oxide/propylene oxide, butylene glycol/adipic acid, and the like. Preferred release rate modification agents include, but are not limited to, dimethyl citrate, triethyl citrate, ethyl heptanoate, glycerin, and hexanediol. Suitable pore-forming agents that may be used in the polymer composition include, but are not limited to,

15 sugars such as sucrose and dextrose, salts such as sodium chloride and sodium carbonate, polymers such as hydroxylpropylcellulose, carboxymethylcellulose, polyethylene glycol, and polyvinylpyrrolidone. Solid crystals that will provide a defined pore size, such as salt or sugar, are preferred.

In specific preferred embodiments the polypeptide, polynucleotide, and antibody

20 compositions of the invention are formulated using the BEMA™ BioErodible Mucoadhesive System, MCA™ MucoCutaneous Absorption System, SMP™ Solvent MicroParticle System, or BCP™ BioCompatible Polymer System of Atrix Laboratories, Inc. (Fort Collins, Colorado).

Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (*see* generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the*

25 *Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. (USA)* 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci.(USA)* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and

30 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et

35 al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention.

Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

5 The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG (e.g., THERACYS®), MPL and nonviable preparations of *Corynebacterium parvum*. In a specific embodiment, Therapeutics of the invention are
10 administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the
15 Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but
20 simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

25 The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, and/or therapeutic treatments described below. Combinations may be administered either concomitantly,
30 e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first,
35 followed by the second.

 In one embodiment, the Therapeutics of the invention are administered in combination with an anticoagulant. Anticoagulants that may be administered with the compositions of the

invention include, but are not limited to, heparin, low molecular weight heparin, warfarin sodium (e.g., COUMADIN®), dicumarol, 4-hydroxycoumarin, anisindione (e.g., MIRADON™), acenocoumarol (e.g., nicoumalone, SINTHROME™), indan-1,3-dione, phenprocoumon (e.g., MARCUMAR™), ethyl biscoumacetate (e.g., TROMEXAN™), and aspirin. In a specific embodiment, compositions of the invention are administered in combination with heparin and/or warfarin. In another specific embodiment, compositions of the invention are administered in combination with warfarin. In another specific embodiment, compositions of the invention are administered in combination with warfarin and aspirin. In another specific embodiment, compositions of the invention are administered in combination with heparin. In another specific embodiment, compositions of the invention are administered in combination with heparin and aspirin.

In another embodiment, the Therapeutics of the invention are administered in combination with thrombolytic drugs. Thrombolytic drugs that may be administered with the compositions of the invention include, but are not limited to, plasminogen, lys-plasminogen, alpha2-antiplasmin, streptokinae (e.g., KABIKINASE™), antiplasmin (e.g., EMINASE™), tissue plasminogen activator (t-PA, altevase, ACTIVASE™), urokinase (e.g., ABBOKINASE™), sauruplase, (Prourokinase, single chain urokinase), and aminocaproic acid (e.g., AMICAR™). In a specific embodiment, compositions of the invention are administered in combination with tissue plasminogen activator and aspirin.

In another embodiment, the Therapeutics of the invention are administered in combination with antiplatelet drugs. Antiplatelet drugs that may be administered with the compositions of the invention include, but are not limited to, aspirin, dipyridamole (e.g., PERSANTINE™), and ticlopidine (e.g., TICLID™).

In specific embodiments, the use of anti-coagulants, thrombolytic and/or antiplatelet drugs in combination with Therapeutics of the invention is contemplated for the detection, prevention, diagnosis, prognostication, treatment, and/or amelioration of thrombosis, arterial thrombosis, venous thrombosis, thromboembolism, pulmonary embolism, atherosclerosis, myocardial infarction, transient ischemic attack, unstable angina. In specific embodiments, the use of anticoagulants, thrombolytic drugs and/or antiplatelet drugs in combination with Therapeutics of the invention is contemplated for the prevention of occlusion of saphenous grafts, for reducing the risk of periprocedural thrombosis as might accompany angioplasty procedures, for reducing the risk of stroke in patients with atrial fibrillation including nonrheumatic atrial fibrillation, for reducing the risk of embolism associated with mechanical heart valves and or mitral valves disease. Other uses for the therapeutics of the invention, alone or in combination with antiplatelet, anticoagulant, and/or thrombolytic drugs, include, but are not limited to, the prevention of occlusions in extracorporeal devices (e.g., intravascular canulas, vascular access shunts in hemodialysis patients, hemodialysis machines, and cardiopulmonary bypass machines).

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs). NRTIs that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). NNRTIs that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

Additional NRTIs include LODENOSINE™ (F-ddA; an acid-stable adenosine NRTI; Triangle/Abbott); COVIRACIL™ (**emtricitabine**/FTC; structurally related to lamivudine (3TC) but with 3- to 10-fold greater activity *in vitro*; Triangle/Abbott); dOTC (BCH-10652, also structurally related to lamivudine but retains activity against a substantial proportion of lamivudine-resistant isolates; Biochem Pharma); **Adefovir** (refused approval for anti-HIV therapy by FDA; Gilead Sciences); PREVEON® (Adefovir Dipivoxil, the active prodrug of adefovir; its active form is PMEA-pp); **TENOFOVIR™** (bis-POC PMPA, a PMPA prodrug; Gilead); **DAPD/DXG** (active metabolite of DAPD; Triangle/Abbott); **D-D4FC** (related to 3TC, with activity against AZT/3TC-resistant virus); **GW420867X** (Glaxo Wellcome); ZIAGEN™ (abacavir/159U89; Glaxo Wellcome Inc.); CS-87 (3'-azido-2',3'-dideoxyuridine; WO 99/66936); and S-acyl-2-thioethyl (SATE)-bearing prodrug forms of β -L-FD4C and β -L-FddC (WO 98/17281).

Additional NNRTIs include COACTINON™ (**Emivirine**/MKC-442, potent NNRTI of the HEPT class; Triangle/Abbott); **CAPRAVIRINE™** (AG-1549/S-1153, a next generation NNRTI with activity against viruses containing the K103N mutation; Agouron); PNU-142721 (has 20- to 50-fold greater activity than its predecessor delavirdine and is active against K103N mutants; Pharmacia & Upjohn); DPC-961 and DPC-963 (second-generation derivatives of efavirenz, designed to be active against viruses with the K103N mutation; DuPont); GW-420867X (has 25-fold greater activity than HBY097 and is active against K103N mutants; Glaxo Wellcome); **CALANOLIDE A** (naturally occurring agent from the latex tree; active against viruses containing either or both the Y181C and K103N mutations); and Propolis (WO 99/49830).

Additional protease inhibitors include **LOPINAVIR**[™] (ABT378/r; Abbott Laboratories); BMS-232632 (an azapeptide; Bristol-Myers Squibb); TIPRANAVIR[™] (PNU-140690, a non-peptidic dihydropyrrone; Pharmacia & Upjohn); PD-178390 (a nonpeptidic dihydropyrrone; Parke-Davis); **BMS 232632** (an azapeptide; Bristol-Myers Squibb); L-756,423 (an indinavir analog; Merck); DMP-450 (a cyclic urea compound; Avid & DuPont); AG-1776 (a peptidomimetic with *in vitro* activity against protease inhibitor-resistant viruses; Agouron); **VX-175/GW-433908** (phosphate prodrug of amprenavir; Vertex & Glaxo Wellcome); **CGP61755** (Ciba); and AGENERASE[™] (amprenavir; Glaxo Wellcome Inc.).

Additional antiretroviral agents include fusion inhibitors/gp41 binders. Fusion inhibitors/gp41 binders include T-20 (a peptide from residues 643-678 of the HIV gp41 transmembrane protein ectodomain which binds to gp41 in its resting state and prevents transformation to the fusogenic state; Trimeris) and T-1249 (a second-generation fusion inhibitor; Trimeris).

Additional antiretroviral agents include fusion inhibitors/chemokine receptor antagonists. Fusion inhibitors/chemokine receptor antagonists include CXCR4 antagonists such as AMD 3100 (a bicyclam), SDF-1 and its analogs, and ALX40-4C (a cationic peptide), T22 (an 18 amino acid peptide; Trimeris) and the T22 analogs T134 and T140; CCR5 antagonists such as RANTES (9-68), AOP-RANTES, NNY-RANTES, and TAK-779; and CCR5/CXCR4 antagonists such as NSC 651016 (a distamycin analog). Also included are CCR2B, CCR3, and CCR6 antagonists. Chemokine receptor agonists such as RANTES, SDF-1, MIP-1 α , MIP-1 β , etc., may also inhibit fusion.

Additional antiretroviral agents include integrase inhibitors. Integrase inhibitors include dicaffeoylquinic (DFQA) acids; L-chicoric acid (a dicaffeoyltartaric (DCTA) acid); quinalizarin (QLC) and related anthraquinones; **ZINTEVIR**[™] (AR 177, an oligonucleotide that probably acts at cell surface rather than being a true integrase inhibitor; Arondex); and naphthols such as those disclosed in WO 98/50347.

Additional antiretroviral agents include hydroxyurea-like compounds such as **BCX-34** (a purine nucleoside phosphorylase inhibitor; Biocryst); ribonucleotide reductase inhibitors such as **DIDOX**[™] (Molecules for Health); inosine monophosphate dehydrogenase (IMPDH) inhibitors such as **VX-497** (Vertex); and mycophenolic acids such as CellCept (**mycophenolate mofetil**; Roche).

Additional antiretroviral agents include inhibitors of viral integrase, inhibitors of viral genome nuclear translocation such as arylene bis(methylketone) compounds; inhibitors of HIV entry such as AOP-RANTES, NNY-RANTES, RANTES-IgG fusion protein, soluble complexes of RANTES and glycosaminoglycans (GAG), and AMD-3100; nucleocapsid zinc finger inhibitors such as dithiane compounds; targets of HIV Tat and Rev; and pharmacoenhancers such as ABT-

Other antiretroviral therapies and adjunct therapies include cytokines and lymphokines such as MIP-1 α , MIP-1 β , SDF-1 α , IL-2, PROLEUKIN™ (aldesleukin/L2-7001; Chiron), IL-4, IL-10, IL-12, and IL-13; interferons such as IFN- α 2a; antagonists of TNFs, NF κ B, GM-CSF, M-CSF, and IL-10; agents that modulate immune activation such as cyclosporin and prednisone; vaccines such as Remune™ (HIV Immunogen), APL 400-003 (Apollon), recombinant gp120 and fragments, bivalent (B/E) recombinant envelope glycoprotein, rgp120CM235, MN rgp120, SF-2 rgp120, gp120/soluble CD4 complex, Delta JR-FL protein, branched synthetic peptide derived from discontinuous gp120 C3/C4 domain, fusion-competent immunogens, and Gag, Pol, Nef, and Tat vaccines; gene-based therapies such as genetic suppressor elements (GSEs; WO 98/54366), and intrakines (genetically modified CC chemokines targetted to the ER to block surface expression of newly synthesized CCR5 (Yang *et al.*, *PNAS* 94:11567-72 (1997); Chen *et al.*, *Nat. Med.* 3:1110-16 (1997)); antibodies such as the anti-CXCR4 antibody 12G5, the anti-CCR5 antibodies 2D7, 5C7, PA8, PA9, PA10, PA11, PA12, and PA14, the anti-CD4 antibodies Q4120 and RPA-T4, the anti-CCR3 antibody 7B11, the anti-gp120 antibodies 17b, 48d, 447-52D, 257-D, 268-D and 50.1, anti-Tat antibodies, anti-TNF- α antibodies, and monoclonal antibody 33A; aryl hydrocarbon (AH) receptor agonists and antagonists such as TCDD, 3,3',4,4',5-pentachlorobiphenyl, 3,3',4,4'-tetrachlorobiphenyl, and α -naphthoflavone (WO 98/30213); and antioxidants such as γ -L-glutamyl-L-cysteine ethyl ester (γ -GCE; WO 99/56764).

In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in

any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rapamycin, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamethoxazole, and vancomycin.

In other embodiments, the Therapeutics of the invention are administered in combination with immunestimulants. Immunostimulants that may be administered in combination with the Therapeutics of the invention include, but are not limited to, levamisole (e.g., ERGAMISOL™), isoprinosine (e.g. INOSIPLEX™), interferons (e.g. interferon alpha), and interleukins (e.g., IL-2).

In other embodiments, Therapeutics of the invention are administered in combination with immunosuppressive agents. Immunosuppressive agents that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells. Other immunosuppressive agents that may be administered in combination with the Therapeutics of the invention include, but are not limited to, prednisolone, methotrexate,

thalidomide, methoxsalen, rapamycin, leflunomide, mizoribine (BREDININ™), brequinar, deoxyspergualin, and azaspirane (SKF 105685), ORTHOCLONE OKT® 3 (muromonab-CD3), SANDIMMUNE™, NEORAL™, SANGDYA™ (cyclosporine), PROGRAF® (FK506, tacrolimus), CELLCEPT® (mycophenolate mofetil, of which the active metabolite is mycophenolic acid), IMURAN™ (azathioprine), glucocorticosteroids, adrenocortical steroids such as DELTASONE™ (prednisone) and HYDELTRASOL™ (prednisolone), FOLEX™ and MEXATE™ (methotrexate), OXSORALEN-ULTRA™ (methoxsalen) and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

10 In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, ATGAM™ (antithymocyte globulin), and GAMIMUNE™. In a specific embodiment, 15 Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

In certain embodiments, the Therapeutics of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, corticosteroids (e.g. 20 betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, and triamcinolone), nonsteroidal anti-inflammatory drugs (e.g., diclofenac, diflunisal, etodolac, fenoprofen, floctafenine, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meclofenamate, mefenamic acid, meloxicam, nabumetone, naproxen, oxaprozin, phenylbutazone, piroxicam, sulindac, tenoxicam, tiaprofenic acid, and tolmetin.), as well as 25 antihistamines, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, 30 pifoxime, proquazone, proxazole, and tenidap.

In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-angiogenic agent. Anti-angiogenic agents that may be administered with the compositions of the invention include, but are not limited to, Angiostatin (Entremed, Rockville, MD), Troponin-1 (Boston Life Sciences, Boston, MA), anti-Invasive Factor, retinoic 35 acid and derivatives thereof, paclitaxel (Taxol), Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, VEGI, Plasminogen Activator Inhibitor-1, Plasminogen

Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include, but are not limited to, platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, (1991)); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, (1992)); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, (1992)); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, (1990)); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, (1987)); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, (1987)); Bisantrone (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4- chloroanthronilic acid disodium or "CCA"; (Takeuchi et al., Agents

Actions 36:312-316, (1992)); and metalloproteinase inhibitors such as BB94.

Additional anti-angiogenic factors that may also be utilized within the context of the present invention include Thalidomide, (Celgene, Warren, NJ); Angiostatic steroid; AGM-1470 (H. Brem and J. Folkman *J Pediatr. Surg.* 28:445-51 (1993)); an integrin alpha v beta 3 antagonist (C. Storgard et al., *J Clin. Invest.* 103:47-54 (1999)); carboxynaminolimidazole; Carboxyamidotriazole (CAI) (National Cancer Institute, Bethesda, MD); Conbretastatin A-4 (CA4P) (OXiGENE, Boston, MA); Squalamine (Magainin Pharmaceuticals, Plymouth Meeting, PA); TNP-470, (Tap Pharmaceuticals, Deerfield, IL); ZD-0101 AstraZeneca (London, UK); APRA (CT2584); Benefin, Byrostatin-1 (SC339555); CGP-41251 (PKC 412); CM101; Dexrazoxane (ICRF187); DMXAA; Endostatin; Flavopridiol; Genestein; GTE; ImmTher; Iressa (ZD1839); Octreotide (Somatostatin); Panretin; Penacillamine; Photopoint; PI-88; Prinomastat (AG-3340) Purlytin; Suradista (FCE26644); Tamoxifen (Nolvadex); Tazarotene; Tetrathiomolybdate; Xeloda (Capecitabine); and 5-Fluorouracil.

Anti-angiogenic agents that may be administered in combination with the compounds of the invention may work through a variety of mechanisms including, but not limited to, inhibiting proteolysis of the extracellular matrix, blocking the function of endothelial cell-extracellular matrix adhesion molecules, by antagonizing the function of angiogenesis inducers such as growth factors, and inhibiting integrin receptors expressed on proliferating endothelial cells. Examples of anti-angiogenic inhibitors that interfere with extracellular matrix proteolysis and which may be administered in combination with the compositions of the invention include, but are not limited to, AG-3340 (Agouron, La Jolla, CA), BAY-12-9566 (Bayer, West Haven, CT), BMS-275291 (Bristol Myers Squibb, Princeton, NJ), CGS-27032A (Novartis, East Hanover, NJ), Marimastat (British Biotech, Oxford, UK), and Metastat (Aeterna, St-Foy, Quebec). Examples of anti-angiogenic inhibitors that act by blocking the function of endothelial cell-extracellular matrix adhesion molecules and which may be administered in combination with the compositions of the invention include, but are not limited to, EMD-121974 (Merck KgaA Darmstadt, Germany) and Vitaxin (Ixsys, La Jolla, CA/Medimmune, Gaithersburg, MD). Examples of anti-angiogenic agents that act by directly antagonizing or inhibiting angiogenesis inducers and which may be administered in combination with the compositions of the invention include, but are not limited to, Angiozyme (Ribozyme, Boulder, CO), Anti-VEGF antibody (Genentech, S. San Francisco, CA), PTK-787/ZK-225846 (Novartis, Basel, Switzerland), SU-101 (Sugen, S. San Francisco, CA), SU-5416 (Sugen/ Pharmacia Upjohn, Bridgewater, NJ), and SU-6668 (Sugen). Other anti-angiogenic agents act to indirectly inhibit angiogenesis. Examples of indirect inhibitors of angiogenesis which may be administered in combination with the compositions of the invention include, but are not limited to, IM-862 (Cytran, Kirkland, WA), Interferon-alpha, IL-12 (Roche, Nutley, NJ), and Pentosan polysulfate (Georgetown University, Washington, DC).

In particular embodiments, the use of compositions of the invention in combination with

anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of an autoimmune disease, such as for example, an autoimmune disease described herein.

In a particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of arthritis. In a more particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of rheumatoid arthritis.

In another embodiment, the polynucleotides encoding a polypeptide of the present invention are administered in combination with an angiogenic protein, or polynucleotides encoding an angiogenic protein. Examples of angiogenic proteins that may be administered with the compositions of the invention include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2, VEGF-3, epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin-like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

In additional embodiments, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to alkylating agents such as nitrogen mustards (for example, Mechlorethamine, cyclophosphamide, Cyclophosphamide Ifosfamide, Melphalan (L-sarcosine), and Chlorambucil), ethylenimines and methylmelamines (for example, Hexamethylmelamine and Thiotepa), alkyl sulfonates (for example, Busulfan), nitrosoureas (for example, Carmustine (BCNU), Lomustine (CCNU), Semustine (methyl-CCNU), and Streptozocin (streptozotocin)), triazines (for example, Dacarbazine (DTIC; dimethyltriazenoimidazolecarboxamide)), folic acid analogs (for example, Methotrexate (amethopterin)), pyrimidine analogs (for example, Fluorouracil (5-fluorouracil; 5-FU), Floxuridine (fluorodeoxyuridine; FudR), and Cytarabine (cytosine arabinoside)), purine analogs and related inhibitors (for example, Mercaptopurine (6-mercaptopurine; 6-MP), Thioguanine (6-thioguanine; TG), and Pentostatin (2'-deoxycoformycin)), vinca alkaloids (for example, Vinblastine (VLB, vinblastine sulfate)) and Vincristine (vincristine sulfate)), epipodophyllotoxins (for example, Etoposide and Teniposide), antibiotics (for example, Dactinomycin (actinomycin D), Daunorubicin (daunomycin; rubidomycin), Doxorubicin, Bleomycin, Plicamycin (mithramycin), and Mitomycin (mitomycin C), enzymes (for example, L-Asparaginase), biological response modifiers (for example, Interferon-alpha and interferon-alpha-2b), platinum coordination compounds (for example, Cisplatin (cis-DDP) and Carboplatin), anthracenedione (Mitoxantrone), substituted ureas (for example, Hydroxyurea), methylhydrazine derivatives (for example, Procarbazine (N-methylhydrazine; MIH), adrenocorticosteroids (for example, Prednisone), progestins (for example, Hydroxyprogesterone caproate, Medroxyprogesterone,

Medroxyprogesterone acetate, and Megestrol acetate), estrogens (for example, Diethylstilbestrol (DES), Diethylstilbestrol diphosphate, Estradiol, and Ethinyl estradiol), antiestrogens (for example, Tamoxifen), androgens (Testosterone propionate, and Fluoxymesterone), antiandrogens (for example, Flutamide), gonadotropin-releasing hormone analogs (for example, Leuprolide),
5 other hormones and hormone analogs (for example, methyltestosterone, estramustine, estramustine phosphate sodium, chlorotrianisene, and testolactone), and others (for example, dicarbazine, glutamic acid, and mitotane).

In one embodiment, the compositions of the invention are administered in combination with one or more of the following drugs: infliximab (also known as Remicade™ Centocor, Inc.),
10 Trocade (Roche, RO-32-3555), Leflunomide (also known as Arava™ from Hoechst Marion Roussel), Kineret™ (an IL-1 Receptor antagonist also known as Anakinra from Amgen, Inc.)

In a specific embodiment, compositions of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or combination of one or more of the components of CHOP. In one embodiment, the compositions of the invention are
15 administered in combination with anti-CD20 antibodies, human monoclonal anti-CD20 antibodies. In another embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies and CHOP, or anti-CD20 antibodies and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with Rituximab. In a
20 further embodiment, compositions of the invention are administered with Rituximab and CHOP, or Rituximab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with tositumomab. In a further embodiment, compositions of the invention are administered with tositumomab and CHOP, or tositumomab and any combination of
25 one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. The anti-CD20 antibodies may optionally be associated with radioisotopes, toxins or cytotoxic prodrugs.

In another specific embodiment, the compositions of the invention are administered in combination Zevalin™. In a further embodiment, compositions of the invention are administered
30 with Zevalin™ and CHOP, or Zevalin™ and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. Zevalin™ may be associated with one or more radisotopes. Particularly preferred isotopes are ⁹⁰Y and ¹¹¹In.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the
35 invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the

invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

5 In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International
10 Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693),
15 TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the
20 Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PIGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-
25 2 (PIGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial
30 Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned
35 references are herein incorporated by reference in their entireties.

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered

with the Therapeutics of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

5 In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, granulocyte macrophage colony stimulating factor (GM-CSF) (sargramostim, LEUKINE™, PROKINE™), granulocyte colony stimulating factor (G-CSF) (filgrastim, NEUPOGEN™), macrophage colony stimulating factor (M-CSF, CSF-1) erythropoietin (epoetin alfa, EPOGEN™, PROCRIT™), stem
10 cell factor (SCF, c-kit ligand, steel factor), megakaryocyte colony stimulating factor, PIXY321 (a GMCSF/IL-3 fusion protein), interleukins, especially any one or more of IL-1 through IL-12, interferon-gamma, or thrombopoietin.

In certain embodiments, Therapeutics of the present invention are administered in combination with adrenergic blockers, such as, for example, acebutolol, atenolol, betaxolol,
15 bisoprolol, carteolol, labetalol, metoprolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, and timolol.

In another embodiment, the Therapeutics of the invention are administered in combination with an antiarrhythmic drug (e.g., adenosine, amiodarone, bretylium, digitalis, digoxin, digitoxin, diltiazem, disopyramide, esmolol, flecainide, lidocaine, mexiletine, moricizine, phenytoin,
20 procainamide, N-acetyl procainamide, propafenone, propranolol, quinidine, sotalol, tocainide, and verapamil).

In another embodiment, the Therapeutics of the invention are administered in combination with diuretic agents, such as carbonic anhydrase-inhibiting agents (e.g., acetazolamide, dichlorophenamide, and methazolamide), osmotic diuretics (e.g., glycerin, isosorbide, mannitol,
25 and urea), diuretics that inhibit Na⁺-K⁺-2Cl⁻ symport (e.g., furosemide, bumetanide, azosemide, piretanide, tripamide, ethacrynic acid, muzolimine, and torsemide), thiazide and thiazide-like diuretics (e.g., bendroflumethiazide, benzthiazide, chlorothiazide, hydrochlorothiazide, hydroflumethiazide, methyclothiazide, polythiazide, trichormethiazide, chlorthalidone, indapamide, metolazone, and quinethazone), potassium sparing diuretics (e.g., amiloride and
30 triamterene), and mineralcorticoid receptor antagonists (e.g., spironolactone, canrenone, and potassium canrenoate).

In one embodiment, the Therapeutics of the invention are administered in combination with treatments for endocrine and/or hormone imbalance disorders. Treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, ¹²⁷I, radioactive isotopes of
35 iodine such as ¹³¹I and ¹²³I; recombinant growth hormone, such as HUMATROPE™ (recombinant somatropin); growth hormone analogs such as PROTROPIN™ (somatrem); dopamine agonists

such as PARLODEL™ (bromocriptine); somatostatin analogs such as SANDOSTATIN™ (octreotide); gonadotropin preparations such as PREGNYL™, A.P.L.™ and PROFASI™ (chorionic gonadotropin (CG)), PERGONAL™ (menotropins), and METRODIN™ (urofollitropin (uFSH)); synthetic human gonadotropin releasing hormone preparations such as FACTREL™ and
5 LUTREPULSE™ (gonadorelin hydrochloride); synthetic gonadotropin agonists such as LUPRON™ (leuprolide acetate), SUPPRELIN™ (histrelin acetate), SYNAREL™ (nafarelin acetate), and ZOLADEX™ (goserelin acetate); synthetic preparations of thyrotropin-releasing hormone such as RELEFACT TRH™ and THYPINONE™ (protirelin); recombinant human TSH such as THYROGEN™; synthetic preparations of the sodium salts of the natural isomers of
10 thyroid hormones such as L-T₄™, SYNTHROID™ and LEVOTHROID™ (levothyroxine sodium), L-T₃™, CYTOMEL™ and TRIOSTAT™ (liothyroine sodium), and THYROLAR™ (liotrix); antithyroid compounds such as 6-*n*-propylthiouracil (propylthiouracil), 1-methyl-2-mercaptoimidazole and TAPAZOLE™ (methimazole), NEO-MERCAZOLE™ (carbimazole); beta-adrenergic receptor antagonists such as propranolol and esmolol; Ca²⁺ channel blockers;
15 dexamethasone and iodinated radiological contrast agents such as TELEPAQUE™ (iopanoic acid) and ORAGRAFIN™ (sodium ipodate).

Additional treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, estrogens or conjugated estrogens such as ESTRACE™ (estradiol), ESTINYL™ (ethinyl estradiol), PREMARIN™, ESTRATAB™, ORTHO-EST™, OGEN™ and estropipate
20 (estrone), ESTROVIS™ (quinestrol), ESTRADERM™ (estradiol), DELESTROGEN™ and VALERGEN™ (estradiol valerate), DEPO-ESTRADIOL CYPIONATE™ and ESTROJECT LA™ (estradiol cypionate); antiestrogens such as NOLVADEX™ (tamoxifen), SEROPHENE™ and CLOMID™ (clomiphene); progestins such as DURALUTIN™ (hydroxyprogesterone caproate), MPA™ and DEPO-PROVERA™ (medroxyprogesterone acetate), PROVERA™ and CYCRIN™
25 (MPA), MEGACE™ (megestrol acetate), NORLUTIN™ (norethindrone), and NORLUTATE™ and AYGESTIN™ (norethindrone acetate); progesterone implants such as NORPLANT SYSTEM™ (subdermal implants of norgestrel); antiprogestins such as RU 486™ (mifepristone); hormonal contraceptives such as ENOVID™ (norethynodrel plus mestranol), PROGESTASERT™ (intrauterine device that releases progesterone), LOESTRIN™, BREVICON™, MODICON™,
30 GENORA™, NELONA™, NORINYL™, OVACON-35™ and OVACON-50™ (ethinyl estradiol/norethindrone), LEVLEN™, NORDETTE™, TRI-LEVLEN™ and TRIPHASIL-21™ (ethinyl estradiol/levonorgestrel) LO/OVRAL™ and OVRAL™ (ethinyl estradiol/norgestrel), DEMULEN™ (ethinyl estradiol/ethynodiol diacetate), NORINYL™, ORTHO-NOVUM™, NORETHIN™, GENORA™, and NELOVA™ (norethindrone/mestranol), DESOGEN™ and
35 ORTHO-CEPT™ (ethinyl estradiol/desogestrel), ORTHO-CYCLEN™ and ORTHO-

TRICYCLEN™ (ethinyl estradiol/norgestimate), MICRONOR™ and NOR-QD™ (norethindrone), and OVRETTE™ (norgestrel).

Additional treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, testosterone esters such as methenolone acetate and testosterone undecanoate; 5 parenteral and oral androgens such as TESTOJECT-50™ (testosterone), TESTEX™ (testosterone propionate), DELATESTRYL™ (testosterone enanthate), DEPO-TESTOSTERONE™ (testosterone cypionate), DANOCRINE™ (danazol), HALOTESTIN™ (fluoxymesterone), ORETON METHYL™, TESTRED™ and VIRILON™ (methyltestosterone), and OXANDRIN™ (oxandrolone); testosterone transdermal systems such as TESTODERM™; androgen receptor 10 antagonist and 5-alpha-reductase inhibitors such as ANDROCUR™ (cyproterone acetate), EULEXIN™ (flutamide), and PROSCAR™ (finasteride); adrenocorticotrophic hormone preparations such as CORTROSYN™ (cosyntropin); adrenocortical steroids and their synthetic analogs such as ACLOVATE™ (alclometasone dipropionate), CYCLOCORT™ (amcinonide), BECLOVENT™ and VANCERIL™ (beclomethasone dipropionate), CELESTONE™ 15 (betamethasone), BENISONE™ and UTICORT™ (betamethasone benzoate), DIPROSONE™ (betamethasone dipropionate), CELESTONE PHOSPHATE™ (betamethasone sodium phosphate), CELESTONE SOLUSPAN™ (betamethasone sodium phosphate and acetate), BETA-VAL™ and VALISONE™ (betamethasone valerate), TEMOVATE™ (clobetasol propionate), CLODERM™ (clocortolone pivalate), CORTEF™ and HYDROCORTONE™ (cortisol (hydrocortisone)), 20 HYDROCORTONE ACETATE™ (cortisol (hydrocortisone) acetate), LOCOID™ (cortisol (hydrocortisone) butyrate), HYDROCORTONE PHOSPHATE™ (cortisol (hydrocortisone) sodium phosphate), A-HYDROCORT™ and SOLU CORTEF™ (cortisol (hydrocortisone) sodium succinate), WESTCORT™ (cortisol (hydrocortisone) valerate), CORTISONE ACETATE™ (cortisone acetate), DESOWEN™ and TRIDESILON™ (desonide), TOPICORT™ 25 (desoximetasone), DECADRON™ (dexamethasone), DECADRON LA™ (dexamethasone acetate), DECADRON PHOSPHATE™ and HEXADROL PHOSPHATE™ (dexamethasone sodium phosphate), FLORONE™ and MAXIFLOR™ (diflorasone diacetate), FLORINEF ACETATE™ (fludrocortisone acetate), AEROBID™ and NASALIDE™ (flunisolide), FLUONID™ and SYNALAR™ (fluocinolone acetonide), LIDEX™ (fluocinonide), FLUOR-OP™ 30 and FML™ (fluorometholone), CORDRAN™ (flurandrenolide), HALOG™ (halcinonide), HMS LIZUIFILM™ (medrysone), MEDROL™ (methylprednisolone), DEPO-MEDROL™ and MEDROL ACETATE™ (methylprednisone acetate), A-METHAPRED™ and SOLUMEDROL™ (methylprednisolone sodium succinate), ELOCON™ (mometasone furoate), HALDRONE™ (paramethasone acetate), DELTA-CORTEF™ (prednisolone), ECONOPRED™ (prednisolone

acetate), HYDELTRASOL™ (prednisolone sodium phosphate), HYDELTRA-T.B.A™ (prednisolone tebutate), DELTASONE™ (prednisone), ARISTOCORT™ and KENACORT™ (triamcinolone), KENALOG™ (triamcinolone acetonide), ARISTOCORT™ and KENACORT DIACETATE™ (triamcinolone diacetate), and ARISTOSPAN™ (triamcinolone hexacetonide);
5 inhibitors of biosynthesis and action of adrenocortical steroids such as CYTADREN™ (aminoglutethimide), NIZORAL™ (ketoconazole), MODRASTANE™ (trilostane), and METOPIRONE™ (metyrapone); bovine, porcine or human insulin or mixtures thereof; insulin analogs; recombinant human insulin such as HUMULIN™ and NOVOLIN™; oral hypoglycemic agents such as ORAMIDE™ and ORINASE™ (tolbutamide), DIABINESE™ (chlorpropamide),
10 TOLAMIDE™ and TOLINASE™ (tolazamide), DYMELOS™ (acetohexamide), glibenclamide, MICRONASE™, DIBETA™ and GLYNASE™ (glyburide), GLUCOTROL™ (glipizide), and DIAMICRON™ (gliclazide), GLUCOPHAGE™ (metformin), ciglitazone, pioglitazone, and alpha-glucosidase inhibitors; bovine or porcine glucagon; somatostatins such as SANDOSTATIN™ (octreotide); and diazoxides such as PROGLYCEM™ (diazoxide).

15 In an additional embodiment, the Therapeutics of the invention are administered in combination with drugs effective in treating iron deficiency and hypochromic anemias, including but not limited to, ferrous sulfate (iron sulfate, FEOSOL™), ferrous fumarate (e.g., FEOSTAT™), ferrous gluconate (e.g., FERGON™), polysaccharide-iron complex (e.g., NIFEREX™), iron dextran injection (e.g., INFED™), cupric sulfate, pyroxidine, riboflavin, Vitamin B₁₂,
20 cyancobalamin injection (e.g., REDISOL™, RUBRAMIN PC™), hydroxocobalamin, folic acid (e.g., FOLVITE™), leucovorin (folinic acid, 5-CHOH4PteGlu, citrovorum factor) or WELLCOVORIN (Calcium salt of leucovorin), transferrin or ferritin.

In another embodiment, Therapeutics of the invention are administered in combination with vasodilating agents and/or calcium channel blocking agents. Vasodilating agents that may be
25 administered with the Therapeutics of the invention include, but are not limited to, Angiotensin Converting Enzyme (ACE) inhibitors (e.g., papaverine, isoxsuprine, benazepril, captopril, cilazapril, enalapril, enalaprilat, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, spirapril, trandolapril, and nylidrin), and nitrates (e.g., isosorbide dinitrate, isosorbide mononitrate, and nitroglycerin). Examples of calcium channel blocking agents that may be administered in
30 combination with the Therapeutics of the invention include, but are not limited to amlodipine, bepridil, diltiazem, felodipine, flunarizine, isradipine, nicardipine, nifedipine, nimodipine, and verapamil.

In certain embodiments, the Therapeutics of the invention are administered in combination with treatments for gastrointestinal disorders. Treatments for gastrointestinal disorders that may be
35 administered with the Therapeutic of the invention include, but are not limited to, H₂ histamine receptor antagonists (e.g., TAGAMET™ (cimetidine), ZANTAC™ (ranitidine), PEPCID™

(famotidine), and AXIDTM (nizatidine)); inhibitors of H⁺, K⁺ ATPase (e.g., PREVACIDTM (lansoprazole) and PRILOSECTM (omeprazole)); Bismuth compounds (e.g., PEPTO-BISMOLTM (bismuth subsalicylate) and DE-NOLTM (bismuth subcitrate)); various antacids; sucralfate; prostaglandin analogs (e.g. CYTOTECTM (misoprostol)); muscarinic cholinergic antagonists; laxatives (e.g., surfactant laxatives, stimulant laxatives, saline and osmotic laxatives); antidiarrheal agents (e.g., LOMOTILTM (diphenoxylate), MOTOFENTM (diphenoxin), and IMODIUMTM (loperamide hydrochloride)), synthetic analogs of somatostatin such as SANDOSTATINTM (octreotide), antiemetic agents (e.g., ZOFTRANTM (ondansetron), KYTRILTM (granisetron hydrochloride), tropisetron, dolasetron, metoclopramide, chlorpromazine, perphenazine, prochlorperazine, promethazine, thiethylperazine, triflupromazine, domperidone, haloperidol, droperidol, trimethobenzamide, dexamethasone, methylprednisolone, dronabinol, and nabilone); D2 antagonists (e.g., metoclopramide, trimethobenzamide and chlorpromazine); bile salts; chenodeoxycholic acid; ursodeoxycholic acid; and pancreatic enzyme preparations such as pancreatin and pancrelipase.

In additional embodiments, the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Example 14: Method of Treating Decreased Levels of the Polypeptide

The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of polypeptides (including agonists thereto), and/or antibodies of the invention. Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a polypeptide of the present invention in an individual may be treated by administering agonists of said polypeptide. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the agonist (including polypeptides and antibodies of the present invention) to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the agonist for six consecutive days. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 13.

Example 15: Method of Treating Increased Levels of the Polypeptide

The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an antagonist of the

invention (including polypeptides and antibodies of the invention).

In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, due to a variety of etiologies, such as cancer.

For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The antisense polynucleotides of the present invention can be formulated using techniques and formulations described herein (e.g. see Example 13), or otherwise known in the art.

Example 16: Method of Treatment Using Gene Therapy-Ex Vivo

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to

confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

5 Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed
10 and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

 The engineered fibroblasts are then transplanted onto the host, either alone or after having
15 been grown to confluence on cytodex 3 microcarrier beads.

Example 17: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of the Invention

 Another method of gene therapy according to the present invention involves operably
20 associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935 (1989); and Zijlstra et al., *Nature*, 342:435-438 (1989). This
25 method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

 Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the
30 polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence
35 contains the same restriction site as the 3' end of the amplified promoter.

 The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The

digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel, then purified by phenol extraction and ethanol precipitation.

5 In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

10 Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

15 Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na₂ HPO₄, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer
20 containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately 3X10⁶ cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid
25 pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3' end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3' end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5' end and a HindIII site at the 3' end. The CMV promoter and
30 the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension (containing
35 approximately 1.5X10⁶ cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 µF and 250-300 V, respectively. As voltage increases, cell

survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

Example 18: Method of Treatment Using Gene Therapy - In Vivo

Another aspect of the present invention is using *in vivo* gene therapy methods to prevent, treat, and/or ameliorate allergic and/or asthmatic diseases and disorders. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present invention may be operatively linked to (i.e., associated with) a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., Cardiovasc. Res. 35(3):470-479 (1997); Chao et al., Pharmacol. Res. 35(6):517-522 (1997); Wolff, Neuromuscul. Disord. 7(5):314-318 (1997); Schwartz et al., Gene Ther. 3(5):405-411 (1996); Tsurumi et al., Circulation 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow

for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within an animal, including muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be used to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

Example 19: Transgenic Animals

The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e.g., baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993)); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989)); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing

polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound

heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

Example 20: Knock-Out Animals

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (e.g., see Smithies et al., Nature 317:230-234 (1985); Thomas & Capecchi, Cell 51:503-512 (1987); Thompson et al., Cell 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (e.g., see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be

placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and “knock-out” animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

Example 21: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations

and their precursors.

In Vitro Assay- Agonists or antagonists of the invention can be assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the agonists or antagonists of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 10^5 B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5×10^{-5} M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and 10^{-5} dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with 3 H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

In vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of agonists or antagonists of the invention, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with agonists or antagonists of the invention identify the results of the activity of the agonists or antagonists on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with agonist or antagonist is used to indicate whether the agonists or antagonists specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

Likewise, a predicted consequence of increased mature B-cell representation *in vivo* is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and agonists or antagonists-treated mice.

The studies described in this example tested activity of agonists or antagonists of the

invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 22: T Cell Proliferation Assay

5 A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of ^3H -thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 μl /well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 degrees C (1 $\mu\text{g}/\text{ml}$ in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to 10 quadruplicate wells (5×10^4 /well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of agonists or antagonists of the invention (total volume 200 μl). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37 degrees C, plates are spun for 2 min. at 1000 rpm and 100 μl of supernatant is removed and stored -20 degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. 15 Wells are supplemented with 100 μl of medium containing 0.5 μCi of ^3H -thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and incorporation of ^3H -thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative control for the effects of agonists or antagonists of 20 the invention.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

25 ***Example 23: Effect of Agonists or Antagonists of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells***

Dendritic cells are generated by the expansion of proliferating precursors found in the 30 peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as $\text{TNF-}\alpha$, causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, 35 downregulation of $\text{FC}\gamma\text{RII}$, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of agonist or antagonist of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells (10^6 /ml) are treated with increasing concentrations of agonists or antagonists of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e.g., R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increased expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of agonists or antagonists of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. Agonists or antagonists of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated processes (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of 2×10^6 /ml in PBS containing PI at a final concentration of 5 μ g/ml, and then incubated at room temperature for 5 minutes before FACS analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of 5×10^5 cells/ml with increasing concentrations of agonists or antagonists of the invention and under the same conditions, but in the absence of agonists or antagonists. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in the presence of agonist or antagonist of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e.g., R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

Oxidative burst. Purified monocytes are plated in 96-w plate at 2×10^5 cell/well. Increasing concentrations of agonists or antagonists of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRP) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 μ l 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of H_2O_2 produced by the macrophages, a standard curve of a H_2O_2 solution of known molarity is performed for each experiment.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

***Example 24: Suppression of TNF alpha-induced adhesion molecule expression by an Agonist
or Antagonist of the Invention***

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Tumor necrosis factor alpha (TNF-a), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of an agonist or antagonist of the invention to mediate a suppression of TNF-a induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF-a treated ECs when co-stimulated with a member of the FGF family of proteins.

To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO₂. HUVECs are seeded in 96-well plates at concentrations of 1×10^4 cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 ul of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 ul volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and

100 µl of 0.1% paraformaldehyde-PBS(with Ca⁺⁺ and Mg⁺⁺) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10 µl of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 µg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20 µl of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer: 1:5,000 (10^0) > $10^{0.5}$ > 10^{-1} > $10^{-1.5}$. 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNPP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 25: Production Of Polypeptide of the Invention For High-Throughput Screening Assays

The following protocol produces a supernatant containing polypeptide of the present invention to be tested. This supernatant can then be used in the Screening Assays described in Examples 27-34.

First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at 2×10^5 cells/well in .5ml

DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

5 The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8-10, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at
10 RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First,
15 person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degree C for 6 hours.

20 While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or HGS CHO-5 media (116.6 mg/L of CaCl₂ (anhyd); 0.00130 mg/L CuSO₄·5H₂O; 0.050 mg/L of Fe(NO₃)₃·9H₂O; 0.417 mg/L of FeSO₄·7H₂O; 311.80 mg/L of KCl; 28.64 mg/L of MgCl₂; 48.84 mg/L of MgSO₄; 6995.50 mg/L of NaCl; 2400.0 mg/L of NaHCO₃; 62.50 mg/L of NaH₂PO₄·H₂O; 71.02 mg/L of Na₂HPO₄; .4320 mg/L of ZnSO₄·7H₂O; .002 mg/L of
25 Arachidonic Acid ; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitric Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine-H₂O; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL-H₂O; 31.29 mg/ml of L-
30 Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL-H₂O; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2H₂O; and 99.65 mg/ml of L-

Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; 0.680 mg/L of Vitamin B₁₂; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal Acetate. Adjust osmolarity to 327 mOsm) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person B adds 1.5ml appropriate media to each well. Incubate at 37 degree C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 27-33.

It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide of the present invention directly (e.g., as a secreted protein) or by polypeptide of the present invention inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

Example 26: Construction of GAS Reporter Construct

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T

helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

5 The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase (“Jaks”) family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

10 The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, *Ann. Rev. Biochem.* 64:621-51 (1995)). A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN-a, IFN-g, and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xaa-Trp-Ser (SEQ ID NO: 2)).

15 Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway. Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway (See Table below). Thus, by using GAS elements

20 linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

<u>Ligand</u>	<u>tyk2</u>	<u>JAKs</u> <u>Jak1</u>	<u>Jak2</u>	<u>Jak3</u>	<u>STATS</u>	<u>GAS(elements) or ISRE</u>
<u>IFN family</u>						
IFN-a/B	+	+	-	-	1,2,3	ISRE
IFN-g		+	+	-	1	GAS (IRF1>Lys6>IFP)
Il-10	+	?	?	-	1,3	
<u>gp130 family</u>						
IL-6 (Pleiotropic)	+	+	+	?	1,3	GAS (IRF1>Lys6>IFP)
Il-11(Pleiotropic)	?	+	?	?	1,3	
OnM(Pleiotropic)	?	+	+	?	1,3	
LIF(Pleiotropic)	?	+	+	?	1,3	
CNTF(Pleiotropic)	-/+	+	+	?	1,3	
G-CSF(Pleiotropic)	?	+	?	?	1,3	
IL-12(Pleiotropic)	+	-	+	+	1,3	
<u>g-C family</u>						
IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
IL-4 (lymph/myeloid)	-	+	-	+	6	GAS (IRF1 = IFP
>>Ly6)(IgH)						
IL-7 (lymphocytes)	-	+	-	+	5	GAS
IL-9 (lymphocytes)	-	+	-	+	5	GAS
IL-13 (lymphocyte)	-	+	?	?	6	GAS
IL-15	?	+	?	+	5	GAS
<u>gp140 family</u>						
IL-3 (myeloid)	-	-	+	-	5	GAS (IRF1>IFP>>Ly6)
IL-5 (myeloid)	-	-	+	-	5	GAS
GM-CSF (myeloid)	-	-	+	-	5	GAS
<u>Growth hormone family</u>						
GH	?	-	+	-	5	
PRL	?	+/-	+	-	1,3,5	
EPO	?	-	+	-	5	GAS(B-
CAS>IRF1=IFP>>Ly6)						
<u>Receptor Tyrosine Kinases</u>						
EGF	?	+	+	-	1,3	GAS (IRF1)
PDGF	?	+	+	-	1,3	
CSF-1	?	+	+	-	1,3	GAS (not IRF1)

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 27-28, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

5':GCGCCTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO: 3)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTGGCAAAGCCTAGGC:3' (SEQ ID NO: 4)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

5':CTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCATCCCGCCCCTAACTCCGCCCGAGTCCGCCCATCTCCGCCCATGGCTGACTAA TTTTATTTATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAG TGAGGAGGCTTTTGGAGGCCTAGGCTTTTGGAAAAAGCTT:3' (SEQ ID NO: 5)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 27-28.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing EGR and NF-KB promoter sequences are described in Example 29. However, many other promoters can be substituted using the protocols described in this Example. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

Example 27: High-Throughput Screening Assay for T-cell Activity.

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 26. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4⁺ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required number of cells (10^7 per transfection), and resuspend in OPTI-MEM to a final concentration of 10^7 cells/ml. Then add 1ml of 1×10^7 cells in OPTI-MEM to T25 flask and incubate at 37 degree C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentamicin, and 1% Pen-Strep. These cells are treated with supernatants containing polypeptide of the present invention or polypeptide of the present invention induced polypeptides as produced by the protocol described in Example 25.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

5 Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

 After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In
10 addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

 The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates
15 should be covered (using sellophene covers) and stored at -20 degree C until SEAP assays are performed according to Example 30. The plates containing the remaining treated cells are placed at 4 degree C and serve as a source of material for repeating the assay on a specific well if desired.

 As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

20 The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

Example 28: High-Throughput Screening Assay Identifying Myeloid Activity

 The following protocol is used to assess myeloid activity of polypeptide of the present
25 invention by determining whether polypeptide of the present invention proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 26. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

30 To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 26, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest 2×10^7 U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

35 Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM

Na₂HPO₄·7H₂O, 1 mM MgCl₂, and 675 uM CaCl₂. Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degree C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting 1×10^8 cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of 5×10^5 cells/ml. Plate 200 ul cells per well in the 96-well plate (or 1×10^5 cells/well).

Add 50 ul of the supernatant prepared by the protocol described in Example 25. Incubate at 37 degree C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 30.

Example 29: High-Throughput Screening Assay for T-cell Activity

NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF- KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 25. Activators or inhibitors of NF-KB would be useful in detecting, preventing, diagnosing, prognosticating, treating, and/or ameliorating diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTCCCC) (SEQ ID NO: 8), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGGACTTT
CCATCCTGCCATCTCAATTAG:3' (SEQ ID NO: 9)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5 5':GCGGCAAGCTTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO: 4)

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene) Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

10 5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGGACTTTCCATC
TGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCCATCCCGCCCCCT
AACTCCGCCCCAGTTCCGCCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTTTATTTATG
CAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTT
TGGAGGCCTAGGCTTTTGCAAAAAGCTT:3' (SEQ ID NO: 10)

15 Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

20 In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes Sall and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with Sall and NotI.

25 Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 27. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 27. As a positive control, exogenous TNF alpha (0.1,1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

30 *Example 30: Assay for SEAP Activity*

As a reporter molecule for the assays described in Examples 27-29, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

35 Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the Table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on a luminometer, thus one should treat 5 plates at each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

10

Reaction Buffer Formulation:

# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6
23	125	6.25
24	130	6.5
25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5

49	255	12.75
50	260	13

Example 31: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability

5 Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other
10 small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

15 For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO₂ incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

20 A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37 degrees C in a CO₂ incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

25 For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10⁶ cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1x10⁶ cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley Cell Wash with 200 ul, followed by an aspiration step to 100 ul final volume.

30 For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-4. The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul.
35 Increased emission at 530 nm indicates an extracellular signaling event caused by the a molecule, either polypeptide of the present invention or a molecule induced by polypeptide of the present

invention, which has resulted in an increase in the intracellular Ca^{++} concentration.

Example 32: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity

5 The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

10 Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine
15 superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, identifying whether polypeptide of the present invention or a molecule induced by polypeptide of the present invention is capable of activating tyrosine kinase signal transduction pathways is of interest. Therefore, the following protocol is designed to identify such molecules
20 capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50
25 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford,MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48
30 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford,MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by
35 incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 25, the medium was removed and 100

ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na₃VO₄, 2 mM Na₄P₂O₇ and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN)) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4°C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degree C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg₂⁺ (5mM ATP/50mM MgCl₂), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl₂, 5 mM MnCl₂, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degree C for 2 min. Initiate the reaction by adding 10ul of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degree C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degree C for one hour. Wash the well as above.

Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

Example 33: High-Throughput Screening Assay Identifying Phosphorylation Activity

As a potential alternative and/or complement to the assay of protein tyrosine kinase activity described in Example 32, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degree C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 25 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation by polypeptide of the present invention or a molecule induced by polypeptide of the present invention.

Example 34: Assay for the Stimulation of Bone Marrow CD34+ Cell Proliferation

This assay is based on the ability of human CD34+ to proliferate in the presence of hematopoietic growth factors and evaluates the ability of isolated polypeptides expressed in mammalian cells to stimulate proliferation of CD34+ cells.

It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond. Therefore, to test the

effect of polypeptides on hematopoietic activity of a wide range of progenitor cells, the assay contains a given polypeptide in the presence or absence of other hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested polypeptide has a stimulatory effect on hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low level of cycling cells, it is likely that any inhibitory effect of a given polypeptide, or agonists or antagonists thereof, might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in cells that are first subjected to *in vitro* stimulation with SCF+IL-3, and then contacted with the compound that is being evaluated for inhibition of such induced proliferation.

Briefly, CD34+ cells are isolated using methods known in the art. The cells are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one hour. The cell count is adjusted to 2.5×10^5 cells/ml. During this time, 100 μ l of sterile water is added to the peripheral wells of a 96-well plate. The cytokines that can be tested with a given polypeptide in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one hour, 10 μ l of prepared cytokines, 50 μ l of the supernatants prepared in Example 25 (supernatants at 1:2 dilution = 50 μ l) and 20 μ l of diluted cells are added to the media which is already present in the wells to allow for a final total volume of 100 μ l. The plates are then placed in a 37°C/5% CO₂ incubator for five days.

Eighteen hours before the assay is harvested, 0.5 μ Ci/well of [3H] Thymidine is added in a 10 μ l volume to each well to determine the proliferation rate. The experiment is terminated by harvesting the cells from each 96-well plate to a filtermat using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60 μ l Microscint is added to each well and the plate sealed with TopSeal-A press-on sealing film. A bar code 15 sticker is affixed to the first plate for counting. The sealed plates are then loaded and the level of radioactivity determined via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

The studies described in this example test the activity of a given polypeptide to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof. As a nonlimiting example, potential antagonists

tested in this assay would be expected to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and a given polypeptide. In contrast, potential agonists tested in this assay would be expected to enhance cell proliferation and/or to decrease the inhibition of cell proliferation in the presence of cytokines and a given polypeptide.

The ability of a gene to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to the gene are useful for the detection, prevention, diagnosis, prognostication, treatment, and/or amelioration of disorders affecting the immune system and hematopoiesis. Representative uses are described in the “Immune Activity” and “Infectious Disease” sections above, and elsewhere herein.

Example 35: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100 μ l of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 μ l volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 μ l of 0.1% paraformaldehyde-PBS(with Ca⁺⁺ and Mg⁺⁺) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10 μ l of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 μ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20 μ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution, referred to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of

glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer: 1:5,000 (10^0) > $10^{-0.5}$ > 10^{-1} > $10^{-1.5}$. 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNPP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

Example 36: Alamar Blue Endothelial Cells Proliferation Assay

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration. Dilutions of the protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37 degreesC overnight. After the overnight incubation of the cells, the growth media is removed and replaced with GIBCO EC-SFM. The cells are treated with the appropriate dilutions of the protein of interest or control protein sample(s) (prepared in SFM) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days. After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form (i.e., stimulated proliferation will produce a stronger signal and inhibited proliferation will produce a weaker signal and the total signal is

proportional to the total number of cells as well as their metabolic activity). The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

5 ***Example 37: Detection of Inhibition of a Mixed Lymphocyte Reaction***

This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by gene products (e.g., isolated polypeptides). Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of
10 cytokine production by accessory cells. Multiple cells may be targeted by these polypeptides since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

Polypeptides of interest found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not
15 limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

Briefly, PBMCs from human donors are purified by density gradient centrifugation using
20 Lymphocyte Separation Medium (LSM[®], density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to 2×10^6 cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to 2×10^5 cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of test materials (50 μ l) is
25 added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1 μ g/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10 μ g/ml. Cells are cultured for 7-8 days at 37°C in 5% CO₂, and 1 μ C of [³H] thymidine is added to wells for the last 16 hrs of culture. Cells are
30 harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances
35 proliferation of lymphocytes.

One skilled in the art could easily modify the exemplified studies to test the activity of

polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

Example 38: Assays for Protease Activity

5 The following assay may be used to assess protease activity of the polypeptides of the invention.

 Gelatin and casein zymography are performed essentially as described (Heusen et al., *Anal. Biochem.*, 102:196-202 (1980); Wilson et al., *Journal of Urology*, 149:653-658 (1993)). Samples are run on 10% polyacryamide/0.1% SDS gels containing 1% gelatin or casein, soaked in 10 2.5% triton at room temperature for 1 hour, and in 0.1M glycine, pH 8.3 at 37°C 5 to 16 hours. After staining in amido black areas of proteolysis appear as clear areas against the blue-black background. Trypsin (Sigma T8642) is used as a positive control.

 Protease activity is also determined by monitoring the cleavage of n-a-benzoyl-L-arginine ethyl ester (BAEE) (Sigma B-4500. Reactions are set up in (25mMNaPO₄, 1mM EDTA, and 1mM 15 BAEE), pH 7.5. Samples are added and the change in adsorbance at 260nm is monitored on the Beckman DU-6 spectrophotometer in the time-drive mode. Trypsin is used as a positive control.

 Additional assays based upon the release of acid-soluble peptides from casein or hemoglobin measured as adsorbance at 280 nm or colorimetrically using the Folin method are performed as described in Bergmeyer, et al., *Methods of Enzymatic Analysis*, 5 (1984). Other 20 assays involve the solubilization of chromogenic substrates (Ward, *Applied Science*, 251-317 (1983)).

Example 39: Identifying Serine Protease Substrate Specificity

 Methods known in the art or described herein may be used to determine the substrate 25 specificity of the polypeptides of the present invention having serine protease activity. A preferred method of determining substrate specificity is by the use of positional scanning synthetic combinatorial libraries as described in GB 2 324 529 (incorporated herein in its entirety).

Example 40: Ligand Binding Assays

30 The following assay may be used to assess ligand binding activity of the polypeptides of the invention.

 Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. The purified ligand for a polypeptide is radiolabeled to high specific activity (50-2000 Ci/mmol) for binding studies. A determination is then made that 35 the process of radiolabeling does not diminish the activity of the ligand towards its polypeptide. Assay conditions for buffers, ions, pH and other modulators such as nucleotides are optimized to establish a workable signal to noise ratio for both membrane and whole cell polypeptide sources.

For these assays, specific polypeptide binding is defined as total associated radioactivity minus the radioactivity measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand is used to define residual nonspecific binding.

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Example 41: Functional Assay in *Xenopus* Oocytes

Capped RNA transcripts from linearized plasmid templates encoding the polypeptides of the invention are synthesized in vitro with RNA polymerases in accordance with standard procedures. In vitro transcripts are suspended in water at a final concentration of 0.2 mg/ml. Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocyte) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual *Xenopus oocytes* in response to polypeptides and polypeptide agonist exposure. Recordings are made in Ca²⁺ free Barth's medium at room temperature. The *Xenopus* system can be used to screen known ligands and tissue/cell extracts for activating ligands.

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Example 42: Microphysiometric Assays

Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, Calif.). The CYTOSENSOR is thus capable of detecting the activation of polypeptide which is coupled to an energy utilizing intracellular signaling pathway.

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Example 43: Extract/Cell Supernatant Screening

A large number of mammalian receptors exist for which there remains, as yet, no cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligands banks as identified to date. Accordingly, the polypeptides of the invention can also be functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, etc., functional screens) against tissue extracts to identify its natural ligands. Extracts that produce positive functional responses can be sequentially subfractionated until an activating ligand is isolated and identified.

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Example 44: Calcium and cAMP Functional Assays

Seven transmembrane receptors which are expressed in HEK 293 cells have been shown to be coupled functionally to activation of PLC and calcium mobilization and/or cAMP stimulation or inhibition. Basal calcium levels in the HEK 293 cells in receptor-transfected or vector control cells were observed to be in the normal, 100 nM to 200 nM, range. HEK 293 cells expressing

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recombinant receptors are loaded with fura 2 and in a single day >150 selected ligands or tissue/cell extracts are evaluated for agonist induced calcium mobilization. Similarly, HEK 293 cells expressing recombinant receptors are evaluated for the stimulation or inhibition of cAMP production using standard cAMP quantitation assays. Agonists presenting a calcium transient or cAMP fluctuation are tested in vector control cells to determine if the response is unique to the transfected cells expressing receptor.

Example 45: ATP-binding assay

The following assay may be used to assess ATP-binding activity of polypeptides of the invention.

ATP-binding activity of the polypeptides of the invention may be detected using the ATP-binding assay described in U.S. Patent 5,858,719, which is herein incorporated by reference in its entirety. Briefly, ATP-binding to polypeptides of the invention is measured via photoaffinity labeling with 8-azido-ATP in a competition assay. Reaction mixtures containing 1 mg/ml of the ABC transport protein of the present invention are incubated with varying concentrations of ATP, or the non-hydrolyzable ATP analog adenylyl-5'-imidodiphosphate for 10 minutes at 4°C. A mixture of 8-azido-ATP (Sigma Chem. Corp., St. Louis, MO.) plus 8-azido-ATP (³²P-ATP) (5 mCi/μmol, ICN, Irvine CA.) is added to a final concentration of 100 μM and 0.5 ml aliquots are placed in the wells of a porcelain spot plate on ice. The plate is irradiated using a short wave 254 nm UV lamp at a distance of 2.5 cm from the plate for two one-minute intervals with a one-minute cooling interval in between. The reaction is stopped by addition of dithiothreitol to a final concentration of 2mM. The incubations are subjected to SDS-PAGE electrophoresis, dried, and autoradiographed. Protein bands corresponding to the particular polypeptides of the invention are excised, and the radioactivity quantified. A decrease in radioactivity with increasing ATP or adenylyl-5'-imidodiphosphate provides a measure of ATP affinity to the polypeptides.

Example 46: Small Molecule Screening

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and polypeptide of the invention.

Thus, the present invention provides methods of screening for drugs or any other agents

which affect activities mediated by the polypeptides of the invention. These methods comprise contacting such an agent with a polypeptide of the invention or fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically
5 labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the invention, and is described in great detail
10 in European Patent Application 84/03564, published on September 13, 1984, which is herein incorporated by reference in its entirety. Briefly stated, large numbers of different small molecule test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with polypeptides of the invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto
15 plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the
20 antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

Example 47: Phosphorylation Assay

In order to assay for phosphorylation activity of the polypeptides of the invention, a
25 phosphorylation assay as described in U.S. Patent 5,958,405 (which is herein incorporated by reference) is utilized. Briefly, phosphorylation activity may be measured by phosphorylation of a protein substrate using gamma-labeled ^{32}P -ATP and quantitation of the incorporated radioactivity using a gamma radioisotope counter. The polypeptides of the invention are incubated with the protein substrate, ^{32}P -ATP, and a kinase buffer. The ^{32}P incorporated into the substrate is then
30 separated from free ^{32}P -ATP by electrophoresis, and the incorporated ^{32}P is counted and compared to a negative control. Radioactivity counts above the negative control are indicative of phosphorylation activity of the polypeptides of the invention.

Example 48: Detection of Phosphorylation Activity (Activation) of the Polypeptides of the Invention in the Presence of Polypeptide Ligands

35 Methods known in the art or described herein may be used to determine the phosphorylation activity of the polypeptides of the invention. A preferred method of determining

phosphorylation activity is by the use of the tyrosine phosphorylation assay as described in US 5,817,471 (incorporated herein by reference).

Example 49: Identification Of Signal Transduction Proteins That Interact With Polypeptides Of The Present Invention

The purified polypeptides of the invention are research tools for the identification, characterization and purification of additional signal transduction pathway proteins or receptor proteins. Briefly, labeled polypeptides of the invention are useful as reagents for the purification of molecules with which it interacts. In one embodiment of affinity purification, polypeptides of the invention are covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as carcinoma tissues, is passed over the column, and molecules with appropriate affinity bind to the polypeptides of the invention. The protein complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

Example 50: IL-6 Bioassay

To test the proliferative effects of the polypeptides of the invention, the IL-6 Bioassay as described by Marz *et al.* is utilized (*Proc. Natl. Acad. Sci., U.S.A.*, 95:3251-56 (1998), which is herein incorporated by reference). Briefly, IL-6 dependent B9 murine cells are washed three times in IL-6 free medium and plated at a concentration of 5,000 cells per well in 50 μ l, and 50 μ l of the IL-6-like polypeptide is added. After 68 hrs. at 37°C, the number of viable cells is measured by adding the tetrazolium salt thiazolyl blue (MTT) and incubating for a further 4 hrs. at 37°C. B9 cells are lysed by SDS and optical density is measured at 570 nm. Controls containing IL-6 (positive) and no cytokine (negative) are utilized. Enhanced proliferation in the test sample(s) relative to the negative control is indicative of proliferative effects mediated by polypeptides of the invention.

Example 51: Assay for Phosphatase Activity

The following assay may be used to assess serine/threonine phosphatase (PTPase) activity of the polypeptides of the invention.

In order to assay for serine/threonine phosphatase (PTPase) activity, assays can be utilized which are widely known to those skilled in the art. For example, the serine/threonine phosphatase (PSPase) activity is measured using a PSPase assay kit from New England Biolabs, Inc. Myelin basic protein (MyBP), a substrate for PSPase, is phosphorylated on serine and threonine residues with cAMP-dependent Protein Kinase in the presence of [³²P]ATP. Protein serine/threonine phosphatase activity is then determined by measuring the release of inorganic phosphate from 32P-

labeled MyBP.

Example 52: Interaction of Serine/Threonine Phosphatases with other Proteins

The polypeptides of the invention with serine/threonine phosphatase activity as determined in Example 51 are research tools for the identification, characterization and purification of additional interacting proteins or receptor proteins, or other signal transduction pathway proteins. Briefly, labeled polypeptide(s) of the invention is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, polypeptide of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as neural or liver cells, is passed over the column, and molecules with appropriate affinity bind to the polypeptides of the invention. The polypeptides of the invention - complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

Example 53: Assaying for Heparanase Activity

In order to assay for heparanase activity of the polypeptides of the invention, the heparanase assay described by Vlodavsky et al is utilized (Vlodavsky, I., et al., Nat. Med., 5:793-802 (1999)). Briefly, cell lysates, conditioned media or intact cells (1×10^6 cells per 35-mm dish) are incubated for 18 hrs at 37°C, pH 6.2-6.6, with ^{35}S -labeled ECM or soluble ECM derived peak I proteoglycans. The incubation medium is centrifuged and the supernatant is analyzed by gel filtration on a Sepharose CL-6B column (0.9 x 30 cm). Fractions are eluted with PBS and their radioactivity is measured. Degradation fragments of heparan sulfate side chains are eluted from Sepharose 6B at $0.5 < K_{av} < 0.8$ (peak II). Each experiment is done at least three times. Degradation fragments corresponding to "peak II," as described by Vlodavsky et al., is indicative of the activity of the polypeptides of the invention in cleaving heparan sulfate.

Example 54: Immobilization of biomolecules

This example provides a method for the stabilization of polypeptides of the invention in non-host cell lipid bilayer constructs (see, e.g., Bieri et al., Nature Biotech 17:1105-1108 (1999), hereby incorporated by reference in its entirety herein) which can be adapted for the study of polypeptides of the invention in the various functional assays described above. Briefly, carbohydrate-specific chemistry for biotinylation is used to confine a biotin tag to the extracellular domain of the polypeptides of the invention, thus allowing uniform orientation upon immobilization. A 50uM solution of polypeptides of the invention in washed membranes is incubated with 20 mM NaIO₄ and 1.5 mg/ml (4mM) BACH or 2 mg/ml (7.5mM) biotin-

hydrazide for 1 hr at room temperature (reaction volume, 150ul). Then the sample is dialyzed (Pierce Slidealizer Cassett, 10 kDa cutoff; Pierce Chemical Co., Rockford IL) at 4C first for 5 h, exchanging the buffer after each hour, and finally for 12 h against 500 ml buffer R (0.15 M NaCl, 1 mM MgCl₂, 10 mM sodium phosphate, pH7). Just before addition into a cuvette, the sample is
5 diluted 1:5 in buffer ROG50 (Buffer R supplemented with 50 mM octylglucoside).

Example 55: TAQMAN

Quantitative PCR (QPCR). Total RNA from cells in culture are extracted by Trizol separation as recommended by the supplier (LifeTechnologies). (Total RNA is treated with DNase
10 I (Life Technologies) to remove any contaminating genomic DNA before reverse transcription.) Total RNA (50 ng) is used in a one-step, 50ul, RT-QPCR, consisting of Taqman Buffer A (Perkin-Elmer; 50 mM KCl/10 mM Tris, pH 8.3), 5.5 mM MgCl₂, 240 μM each dNTP, 0.4 units RNase inhibitor(Promega), 8%glycerol, 0.012% Tween-20, 0.05% gelatin, 0.3uM primers, 0.1uM probe, 0.025units Amplitaq Gold (Perkin-Elmer) and 2.5 units Superscript II reverse transcriptase (Life
15 Technologies). As a control for genomic contamination, parallel reactions are setup without reverse transcriptase. The relative abundance of (unknown) and 18S RNAs are assessed by using the Applied Biosystems Prism 7700 Sequence Detection System (Livak, K. J., Flood, S. J., Marmaro, J., Giusti, W. & Deetz, K. (1995) PCR Methods Appl. 4, 357-362). Reactions are carried out at 48°C for 30 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15s, 60°C for 1
20 min. Reactions are performed in triplicate.

Primers (f & r) and FRET probes sets are designed using Primer Express Software (Perkin-Elmer). Probes are labeled at the 5'-end with the reporter dye 6-FAM and on the 3'-end with the quencher dye TAMRA (Biosource International, Camarillo, CA or Perkin-Elmer).

Example 56: Assays for Metalloproteinase Activity

Metalloproteinases (EC 3.4.24.-) are peptide hydrolases which use metal ions, such as Zn²⁺, as the catalytic mechanism. Metalloproteinase activity of polypeptides of the present invention can be assayed according to the following methods.

30 Proteolysis of alpha-2-macroglobulin

To confirm protease activity, purified polypeptides of the invention are mixed with the substrate alpha-2-macroglobulin (0.2 unit/ml; Boehringer Mannheim, Germany) in 1x assay buffer (50 mM HEPES, pH 7.5, 0.2 M NaCl, 10 mM CaCl₂, 25 μM ZnCl₂ and 0.05% Brij-35) and incubated at 37°C for 1-5 days. Trypsin is used as positive control. Negative controls contain only
35 alpha-2-macroglobulin in assay buffer. The samples are collected and boiled in SDS-PAGE sample buffer containing 5% 2-mercaptoethanol for 5-min, then loaded onto 8% SDS-

polyacrylamide gel. After electrophoresis the proteins are visualized by silver staining. Proteolysis is evident by the appearance of lower molecular weight bands as compared to the negative control.

Inhibition of alpha-2-macroglobulin proteolysis by inhibitors of metalloproteinases

5 Known metalloproteinase inhibitors (metal chelators (EDTA, EGTA, AND HgCl_2), peptide metalloproteinase inhibitors (TIMP-1 and TIMP-2), and commercial small molecule MMP inhibitors) are used to characterize the proteolytic activity of polypeptides of the invention. The three synthetic MMP inhibitors used are: MMP inhibitor I, [IC_{50} = 1.0 μM against MMP-1 and MMP-8; IC_{50} = 30 μM against MMP-9; IC_{50} = 150 μM against MMP-3]; MMP-3 (stromelysin-1)
10 inhibitor I [IC_{50} = 5 μM against MMP-3], and MMP-3 inhibitor II [K_i = 130 nM against MMP-3]; inhibitors available through Calbiochem, catalog # 444250, 444218, and 444225, respectively). Briefly, different concentrations of the small molecule MMP inhibitors are mixed with purified polypeptides of the invention (50 $\mu\text{g}/\text{ml}$) in 22.9 μl of 1x HEPES buffer (50 mM HEPES, pH 7.5, 0.2 M NaCl, 10 mM CaCl_2 , 25 μM ZnCl_2 and 0.05%Brij-35) and incubated at room temperature
15 (24 °C) for 2-hr, then 7.1 μl of substrate alpha-2-macroglobulin (0.2 unit/ml) is added and incubated at 37°C for 20-hr. The reactions are stopped by adding 4x sample buffer and boiled immediately for 5 minutes. After SDS-PAGE, the protein bands are visualized by silver stain.

Synthetic Fluorogenic Peptide Substrates Cleavage Assay

20 The substrate specificity for polypeptides of the invention with demonstrated metalloproteinase activity can be determined using synthetic fluorogenic peptide substrates (purchased from BACHEM Bioscience Inc). Test substrates include, M-1985, M-2225, M-2105, M-2110, and M-2255. The first four are MMP substrates and the last one is a substrate of tumor necrosis factor- α (TNF- α) converting enzyme (TACE). All the substrates are prepared in 1:1
25 dimethyl sulfoxide (DMSO) and water. The stock solutions are 50-500 μM . Fluorescent assays are performed by using a Perkin Elmer LS 50B luminescence spectrometer equipped with a constant temperature water bath. The excitation λ is 328 nm and the emission λ is 393 nm. Briefly, the assay is carried out by incubating 176 μl 1x HEPES buffer (0.2 M NaCl, 10 mM CaCl_2 , 0.05% Brij-35 and 50 mM HEPES, pH 7.5) with 4 μl of substrate solution (50 μM) at 25 °C for 15
30 minutes, and then adding 20 μl of a purified polypeptide of the invention into the assay cuvet. The final concentration of substrate is 1 μM . Initial hydrolysis rates are monitored for 30-min.

Example 57: Characterization of the cDNA contained in a deposited plasmid

35 The size of the cDNA insert contained in a deposited plasmid may be routinely determined using techniques known in the art, such as PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the cDNA sequence. For example, two primers of 17-30 nucleotides

derived from each end of the cDNA (i.e., hybridizable to the absolute 5' nucleotide or the 3' nucleotide end of the sequence of SEQ ID NO:X, respectively) are synthesized and used to amplify the cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 ul of reaction mixture with 0.5
5 ug of the above cDNA template. A convenient reaction mixture is 1.5-5 mM MgCl₂, 0.01% (w/v) gelatin, 20 uM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis. The
10 PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product. It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

Incorporation by Reference

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. In
20 addition, the sequence listing submitted herewith is incorporated herein by reference in its entirety. The specification and sequence listing of each of the following U.S. and PCT applications are herein incorporated by reference in their entirety (filing dates shown in format "year-month-day" (yyyy-mm-dd)): Application No. 60/278,650 filed on 2001-03-27, Application No. 09/950,082 filed on 2001-09-12, Application No. 09/950,083 filed on 2001-09-12, Application No. 60/306,171
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 2002-03-26.

Applicant's File	International Application
Reference Number: PS953PCT	Number: Unassigned

INDICATIONS RELATING TO DEPOSITED BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited biological material referred to in Table 1A

of the description.

B. IDENTIFICATION OF DEPOSIT:

Name of Depository: **American Type Culture Collection**

Address of Depository: **10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America**

	Accesssion Number	Date of Deposit		Accesssion Number	Date of Deposit
1	203917	April 8, 1999	2	203918	April 8, 1999
3	203957	April 26, 1999	4	203959	April 26, 1999
5	203960	April 26, 1999	6	203979	April 29, 1999
7	PTA-181	June 7, 1999	8	PTA-793	September 27, 1999
9	PTA-794	September 27, 1999	10		